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ACCELERATION OF HEALING BY PRESSURE APPLICATION TO EXPERIMENTAL THERMAL BURNS *

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In a previous paper (Cameron *et al.*, 1945) we described an experimental investigation of some of the effects of applying pressure to thermal burns. We showed that the prompt application of pressure bandages to severely burnt limbs of goats retarded the loss of plasma from blood vessels injured by heat, diminished or prevented the occurrence of, hæmoconcentration and favourably influenced the clinical condition of the animals. We emphasised the need for early action and produced some evidence that delay in treatment beyond four hours after burning gave unfavourable results. These observations were limited to the 24 hours immediately after injury.

In this paper we describe experiments planned to find out (1) whether prompt pressure treatment influences the healing of burns, (2) how long pressure should be maintained in order to get the best results and (3) the effect on healing of delaying pressure treatment. We also give some information about treatment of burns with penicillin cream and Camzol cream (an ointment containing sulphathiazole, zinc oxide and amyl salicylate). The measure of healing adopted in most of these experiments was the time in days taken for complete repair of standard burns.

Methods

1. Adult female goats, 20-40 kg. body weight, were anæsthetised by intravenous administration of Nembutal (15 mg./kg. B.W.), and the closely clipped right forelimb burnt by immersing it for 2½ minutes in water kept at

a steady temperature of 86° C. This method is identical in all respects with that used in our previous work, except that one limb only was burned. The area of burning extended from the hoof to 5 cm. above the knee joint and involved approximately 5 per cent. of the total body surface. Burns were of the same extent and degree in treated and control goats, for animals were carefully selected so as to give pairs of nearly the same body weight and build and technique was strictly adhered to in each pair. Quick-setting Cellona plaster bandages were closely wrapped around the burnt limb, with a considerable overlap beyond the burn margin. A layer of dry sterile gauze separated the plaster from the skin. The bandages were kept on for 30 days. Control goats received no plaster application, but were dressed with dry sterile gauze covered with an ordinary bandage kept in position by a strip of Elastoplast for 30 days. Dressings were thereafter changed at frequent intervals during healing, with both pressure-treated and control animals.

Since the goats were housed in an ordinary shed with a straw covering to the floor, it was essential to control infection, preferably by some means other than local applications of antiseptics. This was done by daily intravenous administration to each goat of soluble sulphathiazole (May and Baker's Thiasamide sodium) in amounts sufficient to maintain a blood sulphonamide level of 3 mg. per cent. Usually a dose of 50 mg./kg. B.W. sufficed, but the amount was checked each week by estimation of the sulphonamide content of each animal's blood and the dose adjusted accordingly. No evidence of sensitisation to sulphonamides was detected.

In addition to following through to completion the healing times of burns in pressure-treated and untreated goats, a series was used for studying the progress of healing. Pairs of animals, one treated with pressure, the other without, were shot at intervals after burning. The weights of the burnt leg and the corresponding normal leg were determined, the burnt tissue dissected off and its weight compared with the corresponding amount of tissue from the normal left forelimb, and sections prepared for histological examination as described in our previous report. Here again, much care was taken to select animals of similar weight and build for comparison at the different stages.

2. In a second series, pressure treatment was initiated at once but the plaster bandage was removed at varying intervals after burning. Thus in a group of 6 goats the plaster was removed after 3 days, in 5 goats after 9 days and in 4 goats after 16 days. For comparison with these we had the 8 goats of the previous experiment from which the plaster had been removed 30 days after burning. In all instances, sulphathiazole was administered intravenously at daily intervals. After removal of the plaster, the burnt limb was simply enclosed in sterile gauze and covered by a bandage secured with Elastoplast; this was renewed at frequent intervals. In a few cases with local sepsis, penicillin cream was applied, pus being washed away with sterile saline. Suppuration invariably cleared up in a few days. This complication was confined to animals in which the plaster bandage had been removed 3 days after burning. It was striking how seldom local infection occurred provided the pressure bandages were retained for 9 days or longer.

3. In a third series, pressure treatment was commenced 2 or 4 hours after burning and maintained for 19 days; such animals were treated as in the second series. These also received daily intravenous doses of sulphathiazole.

4. In a fourth series, goats with standard burns were treated with local applications of penicillin cream * and Camzol cream † protected with sterile

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* The penicillin cream was prepared from 120 g. Lanette wax SX, distilled water and 350 units of penicillin per g. of final product.

† Camzol cream is composed of sulphathiazole 5 parts, a calamine preparation 5 parts, zinc oxide 5 parts, amyl salicylate 25 parts, Lanette wax 4 parts, distilled water (60° F.) to 100 parts. We are indebted to Mr T. Truckle for this formula.

gauze coverings but no pressure bandages. This group constituted a further control to the first experiment.

The number of animals in each of these experimental groups was not large, but the results were so consistent that we have little doubt they give a correct picture of what was happening Limited accommodation and the amount of work entailed in the daily inspection and sulphathiazole administration prevented us from dealing with larger numbers, it was our intention to carry out further experiments, but the termination of the European War brought our plans to an end

We assessed healing by (1) the disappearance of all scabs, (2) the return of free mobility of the soft structures in relation to the bony framework, (3) complete epithelisation of the burnt area and (4) resumption of normal function in the affected limb, as shown by the animal's use of the limb in walking A further check was obtained by microscopic examination of selected areas of the burnt region.

Results

1 The prompt application of pressure to a burn and its maintenance for 30 days led to accelerated healing Table I summarises the results for 8 goats so treated, together with 7 control animals kept under identical conditions. The mean healing time for

TABLE I

Effect on healing times of applying pressure bandage immediately after burning

Pressure bandage applied for 30 days		No pressure bandage					
Sulphathiazole I V		Sulphathiazole I V		Penicillin cream		Camzol cream	
Goat	Healing time (days)	Goat	Healing time (days)	Goat	Healing time (days)	Goat	Healing time (days)
184	34	192	38	267	70	261	56
185	34	193	53	268	50	262	70
186	37	194	56	269	60	263	58
187	12	195	59	270	75	264	56
188	42	196	70	271	died	265	58
189	46	197	80	272	55	266	50
190	47	198	85				
191	34						
Mean healing time	39 5 ± 1 8		63 ± 5 7		62		58

the pressure-treated goats was 39·5±1·8 days, for the controls 63± 5·7 days. A significant reduction had therefore resulted. Table I also gives information about goats treated by applying penicillin cream and Camzol cream to the burns, without employing pressure. The mean healing times for these groups were 62 and 58 days respectively, figures which are not far removed from the mean for the other control group

There was no doubt of the beneficial effects of plaster bandaging When the bandages were removed after 30 days the limbs showed little swelling, repair was well advanced and the animals soon used the affected limbs freely. By the 38th day most of these goats

burnt legs and skin. Although we do not wish to place too much
emphasis on the quantitative results, there is no question that
pressure-treated animals throughout this period of observation showed
much lighter burns than the control animals. Histological examination
of the limbs showed that pressure application is associated with less
œdema in and around the burn and less intense leucocytic infiltration
and tendency to abscess formation, whilst healing of epidermis and
dermis is, on the whole, more advanced at corresponding stages,
with the formation of much less reparative tissue.

We conclude from these experiments that pressure treatment of
a burn (1) reduces the severity of local œdema, (2) accelerates the
rate of healing, (3) diminishes the amount of reparative tissue and
(4) probably reduces the tendency to local sepsis.

2. The effect of removing plaster bandages from burns at various
intervals is clearly shown in table III. The mean healing time for

TABLE III

*Effect on healing times of removing pressure bandages at
varying intervals after burning*

Pressure bandage removed after							
3 days		9 days		16 days		30 days	
Goat	Healing time (days)	Goat	Healing time (days)	Goat	Healing time (days)	Goat	Healing time (days)
243	70	249	42	255	42	184	34
244	70	251	42	256	46	185	34
245	70	252	46	258	40	186	37
246	60	253	44	260	36	187	42
247	70	254	42			188	42
248	60					189	46
						190	47
						191	34
Mean healing time	66·7		43		41		39·5

burns in which the pressure bandages were kept on for 3 days only
was 66·7 days. With 9 days' pressure the mean healing time was
43 days, with 16 days' pressure it was 41 days, and with 30 days'
pressure we have seen that healing was judged to be complete on an
average in 39·5 days. It seems that a minimal period of at least
9 days is necessary if the best results are to be obtained from pressure
application. It is doubtful whether prolonged pressure treatment
is advantageous, since there is little difference between the healing
time with 30 days' pressure and that with 9 or 16 days' pressure.

The poor results with restricted pressure treatment (3 days) is
not surprising, for when the plaster was removed after this short
interval severe œdema developed at the burn site within a few hours.
It appeared that vascular equilibrium had not yet been established in

the burn. This effect was independent of the presence of sepsis, for it was equally marked in perfectly clean burns. Similar conclusions have been reached by Glenn *et al.* (1943) from experiments with dogs.

3. In table IV is shown the effect on healing time of delay in applying pressure bandages to burns. With 2 hours' delay the mean

TABLE IV

Effect on healing times of delay in application of pressure bandage

Pressure bandage applied					
immediately after burning *		2 hours after burning †		4 hours after burning †	
Goat	Healing time (days)	Goat	Healing time (days)	Goat	Healing time (days)
184	34	235	54	239	45
185	34	236	54	240	49
186	37	237	45	241	54
187	42	238	54	242	54
188	42				
189	46				
190	47				
191	34				
Mean healing times	39·5		51·5		50·5

* Pressure bandages applied for 30 days.
† Pressure bandages applied for 19 days.

healing time was 51·5 days. Much the same result followed upon a delay of 4 hours (mean healing time 50·5 days). We have included in this table the results for immediate pressure treatment, where a mean healing time of 39·5 days was obtained with burns to which plaster bandages were applied within a few minutes of burning. These results are not strictly comparable, for the plaster had been removed 30 days after application whereas in the other two groups it was removed after 19 days. But our previous experiments indicate that removal of plaster between the 9th and 30th days after burning does not make much difference in healing times, so that this criticism is perhaps not a serious one.

We conclude from this study that the pressure treatment should be initiated as soon as possible after burning if the best healing results are to be obtained. Nevertheless, with a delay of 2 or 4 hours, healing is still favourably influenced, since the mean healing time was reduced from 63 days in goats without pressure to 50·5 days in goats with pressure applied 4 hours after burning.

4. We have already drawn attention to the less favourable healing of burns treated by the local application of penicillin cream or Camzol cream. In both groups mean healing times were almost identical with that in goats treated by intravenous sulphathiazole and gauze dressings.

Discussion

Our experiments show that pressure treatment of extensive second and third degree burns of the extremities of a large animal, the goat, has a beneficial influence on the healing of such burns. Prompt application of plaster bandages has reduced very considerably the mean healing time of standard burns as compared with similar burns simply dressed with sterile gauze enclosed in bandages without pressure. In both instances, chemotherapy was strenuously employed through the agency of daily intravenous administration of sulphathiazole controlled by estimation of the blood sulphonamide level.

Our experiments also suggest that some benefit may be expected even when pressure treatment is delayed for 2 or 3 hours. There seems to be a direct relationship between promptitude of treatment and healing—the sooner pressure is applied the more rapid is the healing of the burn. But there is a limiting time to this rule, for delay beyond 4 hours is unlikely to give good results. The determining factor is most likely the rate at which œdema develops in the burnt area. In our previous report on the effects produced by pressure treatment during the first 24 hours after burning, we gave much evidence that the maximum loss of plasma from the blood vessels of a burnt area, *i.e.* the œdema, takes place within 4 hours of burning. The important period, we felt, is the first half-hour, for plasma loss is extremely pronounced then. When such an area is compressed with plaster, a striking reduction in plasma loss occurs and œdema is greatly diminished. Our more recent experiments have confirmed these conclusions and have added the interesting fact that healing of a compressed burn is much hastened. It is difficult to escape the conclusion that there is a close connection between these facts and that, because of the diminution of local œdema brought about by pressure treatment, healing is favourably influenced. Histological studies have impressed us with the lessened formation of reparative tissue in a healing compressed burn contrasted with an uncompressed burn. This no doubt depends on the amount of fibrin deposited from the plasma set free in and around the burn. If plasma infiltration is reduced fibrin production is diminished, and there is less of this " foreign " material to be removed subsequently by phagocytes and enzyme action. In other words, the inflammatory reaction around the burn will be less pronounced if there is less fibrin present and the burden placed upon the tissues in the course of repair will be lightened. We believe pressure to act in this way, and we are encouraged in this view by the observation that usually there is considerable shrinkage of the burnt tissues 24 hours after applying pressure, a fairly wide gap then existing between the plaster and the skin surface. It is difficult to see how such a bandage can exert much pressure from then on. No doubt it is beneficial in immobilising

the affected limb by acting as a splint and giving support and rest to a part which requires these for uninterrupted healing. It is possible of course that other factors may be concerned, since we have shown that if the plaster be removed a few days after burning, œdema promptly occurs and healing is considerably delayed. A critical phase thus appears to exist for some days following burning, which is counteracted by immobilising the limb in a plaster casing, but we have no clear conception of what underlies this phase.

Our experiments also suggest that there is no point in prolonging the pressure treatment after the critical phase is passed. Much more elaborate experiments would be required to determine with accuracy the optimum time for terminating pressure, but it seems to be somewhere in the region of a week. Glenn *et al.* have expressed similar views based on their experiments with dogs, and we understand that human experience agrees with this conclusion. By this time repair is well in evidence and granulation tissue is forming, whilst new blood vessels are being laid down. It is possible that such vessels may now have acquired a certain degree of stability which enables them to resist the wear and tear of tissue movement so that they can stand up to the increased stresses thrown upon them when the plaster bandage is removed. But here we touch upon a field of pathology about which very little is known, and still less is to be gained by theorising.

Diminished formation of fibrin through the inhibition of œdema by pressure may also be responsible for the lessened incidence of infection which has struck us so forcibly in these experiments. Fibrin is generally regarded as an excellent culture medium for bacteria. If it be reduced in amount, then it is possible that the chances of bacterial growth are also reduced, especially if sulphonamides are circulating in the blood and tissue fluids, as was the case with our animals.

Because of the reduction in inflammatory reaction and the more rapid healing, together with the lessened tendency to infection, it is not surprising that deformity is not so marked in a burnt limb treated with pressure as in one treated without. Adhesions around joints and between tendons were noticeably fewer and finer on dissection and left the goats with no permanent disability.

Finally we would emphasise the need for caution in applying these results to man, for mechanical factors alone are widely divergent in the two species and there may be further variables which affect the conclusions.

Conclusions

1. The prompt application of pressure bandages to thermal burns of the extremities of goats accelerates the rate of healing and greatly reduces the mean healing time.

2. Pressure interferes with effusion of plasma and the formation

of fibrin at the burn site, decreases the amount of reparative tissue and probably lessens the chance of local infection.

3. Pressure should be applied as soon as possible after burning to get the best results. A delay of 4 hours is probably the limit for beneficial effects.

4. There is no need to maintain pressure application for much longer than a week, for there is very little difference in mean healing times of standard burns treated with pressure for 9, 16 or 30 days. Removal of pressure bandages during a critical phase lasting for several days after burning is followed by severe œdema of the burned area and delay in healing.

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HÆMOLYSIN TESTS FOR THE RAPID IDENTIFICATION OF CL. ŒDEMATIENS AND CL. SEPTICUM

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THE cultural and biochemical tests for the identification of pathogenic clostridia are extravagant of time and material, entailing long incubation and numerous test media to distinguish the closely similar non-pathogenic species. In-vitro serological tests, depending on specific toxins provide a quick alternative, since they are usually easy to apply. The Nagler test (Hayward, 1943) provides a ready means of distinguishing *Cl. welchii*. For the two remaining species of the chief gas-gangrene clostridia—*Cl. septicum* and *Cl. œdematiens* —rapid identification by hæmolysins has been investigated. The routine use of antitoxin-controlled hæmolysin tests for the identification of clostridia was proposed by Reed and Orr (1941b), who advocated tests of 24-hour gelatin-thioglycollate broth cultures against rabbit red cells, both with and without "antitoxin". The antitoxin was not specified except as having been prepared against the toxin of a given species. There are, however, many variable factors which must be standardised before a reliable qualitative test can be defined for routine use. For example, where an organism elaborates more than one hæmolysin, the culture medium determines to a large extent the type of hæmolysin produced and growth conditions must be chosen to avoid the production of oxygen-labile lysins, which are antigenically related in *Cl. welchii*, *Cl. tetani* (Todd, 1941) and *Cl. œdematiens* (C. L. Oakley, personal communication). Again, antitoxic sera prepared for the therapy of infections with a given Clostridium do not necessarily contain antibodies specific for the hæmolysins produced by the Clostridium in culture and may contain large amounts of antihæmolysins for other species, since it is common for one animal to be used for the preparation of several different antisera. Finally, the actual hæmolytic test must be performed with careful attention to the preparation of the red cells and to the *p*H of the reacting mixture.

The lysins for human red cells produced by *Cl. œdematiens* and *Cl. septicum* in Brewer's medium (Brewer, 1940) are serologically

species-specific, and by selection of appropriate antisera it has proved possible to identify the two organisms by a hæmolysin test within 48 and sometimes even 24 hours of the receipt of pathological material.

Technical considerations

The medium. Brewer's medium was qualitatively satisfactory, since the *œdematiens* and *septicum* hæmolysins formed in it were completely neutralised by monospecific antisera (see below). In cooked meat medium and in media containing a strip of iron (Hayward and Miles, 1943), *Cl. œdematiens* formed a serologically different hæmolysin unsuitable for the test described below. Brewer's medium may be made with tryptic digest of ox heart or meat infusion containing added peptone and enriched with 5 per cent. of Fildes's extract or

——————— Brewer's medium + 1 per cent. glucose.
– – – – – – Brewer's medium + 0·2 per cent. glucose + 10 per cent. gelatin.
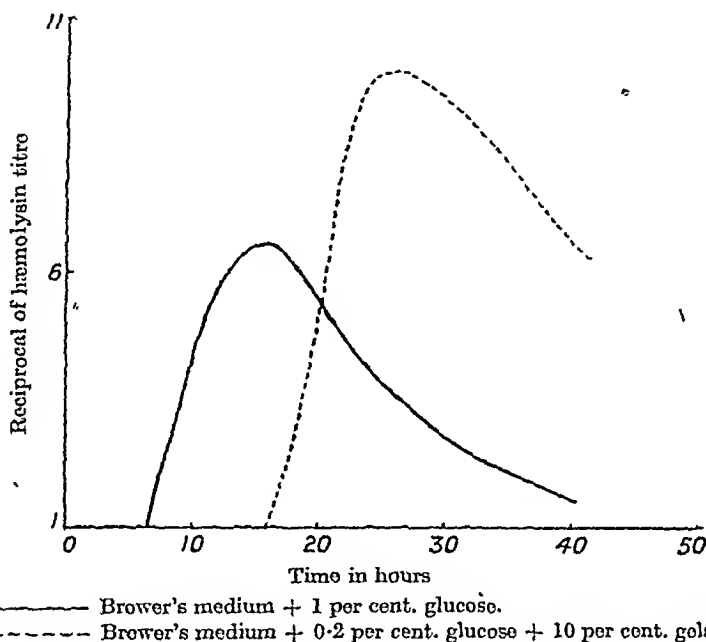
Fig. 1.—Influence of gelatin on production of hæmolysin by *Cl. œdematiens* Cossard.

0·2-1 per cent. of glucose, or with 10 per cent. of gelatin and 0·2 per cent. of glucose as proposed by Reed and Orr (1941b). These media may be incubated in air ; plain nutrient broth is suitable if incubated anaerobically. The *septicum* hæmolysins tested were all serologically similar whether produced in cooked meat medium, Brewer's medium made with either type of broth, plain or enriched with Fildes's extract or 0·2 per cent. glucose, or broth containing a strip of iron.

Cultures in Brewer's medium yielded adequate amounts of hæmolysin in a short time, even from small inocula of fastidious strains of *Cl. œdematiens*. It was reported previously (Hayward, 1941) that Brewer's medium supported the growth only of large inocula of clostridia ; this is true only if the depth of the tubed medium is less than the 7 cm. specified by Brewer, and the conclusion is therefore false. Tests of graded inocula, viable-counted by the method of Miles and Misra (1938), showed that enrichment with glucose or gelatin as suggested by Reed and Orr is unnecessary. The hæmolytic titres of various cultures, defined as the final dilution producing at least 50 per cent. hæmolysis when mixed with a final concentration of 1·5 per cent. washed human red cells,

was as follows :—*Cl. œdematiens*, 1 : 4 to 1 : 10 (five cultures of one strain, one of a second) and *Cl. septicum*, 1 : 12 to 1 : 50 (3 cultures of 2 strains). The amount of hæmolysin produced by *Cl. œdematiens* was not increased by the addition of one per cent. glucose. Ten per cent. " photographic " gelatin increased the hæmolysin titre but delayed its formation by retarding growth in the early stages (fig. 1). The culinary gelatin recommended by Reed and Orr (1941*a*) was unobtainable.

Time relation of hæmolysin production. In Brewer's medium the hæmolysin of *Cl. septicum* forms at a very early stage of growth, when turbidity is barely visible, but it soon disappears and may be absent by the time growth is heavy (fig. 2). Of 44 overnight cultures representing 16 strains of *Cl. septicum*, 40
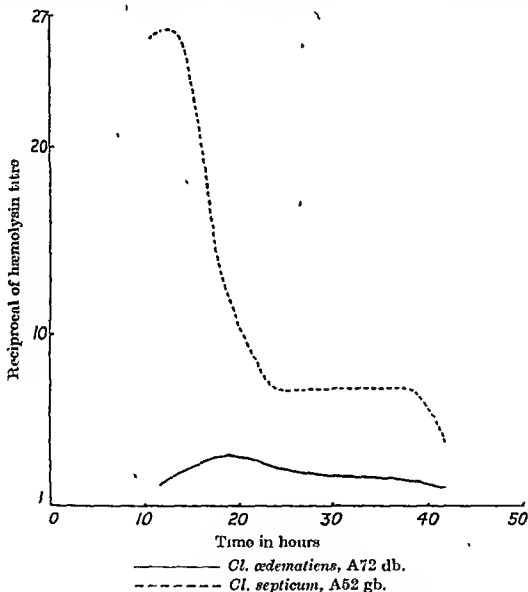
Fig. 2.—Time relations of hæmolysin production.

produced at least 50 per cent. hæmolysis, 3 between 25 and 50 per cent. and 1 less than 25 per cent.; none was non-hæmolytic. The hæmolysin of *Cl. œdematiens* appears later. It never reaches such a high titre as the hæmolysin of *Cl. septicum*, but it is relatively stable and can be found in cultures that are several days old (fig. 2). Of 65 overnight cultures representing 15 strains of *Cl. œdematiens*, 39 produced at least 50 per cent. hæmolysis, 8 between 25 and 50 per cent. and 2 less than 25 per cent.; 16 were non-hæmolytic. Prolonged incubation would probably have yielded positive results, since all these cultures were ultimately proved to be hæmolytic (see *Results*). With an unknown culture, therefore, overnight incubation is the most likely to yield a positive result. Negative results may indicate either a non-hæmolytic strain or a hæmolysin-producer tested at the wrong stage of growth; the test should then be repeated with 6- and 48-hour cultures. If all three cultures, at 6, 24

and 48 hours, are non-hæmolytic, the unknown organism almost certainly does not form a hæmolysin in Brewer's medium.

Hydrogen ion concentration. The *septicum* hæmolysin for human red cells formed in Brewer's medium is fully active only below pH about 6·5. Above this the hæmolysin steadily decreases in activity as the pH rises, and is inactive from pH 7·3 to 7·6. Below pH 5·5-5·6, however, the red cell suspension undergoes acid lysis, so that the test system must be buffered to lie within the range pH 5·8-6·0. Tests made without lowering the pH with a buffer may fail to show the presence of hæmolysin, especially as some of both normal and antitoxic sera apparently buffer the system at a higher pH. Acetate, phosphate and citrate buffers and even weak hydrochloric acid may be used to lower the pH and do not affect the hæmolysin of *Cl. septicum*. $M/20$ acetate buffer of pH 5·2 is strong enough to overcome the buffering effects of serum and culture medium. The antiserum may be diluted with $M/5$ buffer (which is approximately isotonic) so that the final dilution of the buffer in the hæmolytic system is $M/20$ (see table III). In control mixtures without culture acid hæmolysis occurs unless uninoculated medium is used to make up the volume. The *œdematiens* hæmolysin is active at pH 7·0. A standard pH of 6·0 can nevertheless be adopted for all tests, since at this pH neither *œdematiens*, *welchii*, *tetani* nor *chauvoei* hæmolysins are adversely affected in the standard test conditions.

Walbum (1938) has investigated the influence of pH on the hæmolysins for horse red cells formed in cooked meat medium by *Cl. œdematiens* and *Cl. septicum*. His results are not in accordance with those given above and suggest that he was dealing with hæmolysins other than those for human red cells formed in Brewer's medium. It is interesting, however, to note that the point of maximum stability of his *septicum* hæmolysin was pH 5·0 and of his *œdematiens* hæmolysin pH 6·0.

Standardisation of antihæmolysins. The following clostridia from pathological material, being hæmolytic on horse blood agar, were tested in Brewer's medium for human red cell hæmolysins. The figures in brackets indicate the number of strains tested :—*Cl. welchii* (23), *Cl. tetani* (16), *Cl. chauvoei* (2), *Cl. œdematiens* (15), *Cl. septicum* (16), *Cl. bifermentans* (8), *Cl. histolyticum* (3), *Cl. sporogenes* (6), *Cl. hastiforme* (1), and *Cl. sphenoides* (1). Of these only *Cl. welchii*, *Cl. tetani*, *Cl chauvoei*, *Cl. œdematiens* and *Cl. septicum* regularly formed hæmolysins ; occasionally *bifermentans* cultures formed a weak hæmolysin, but this was inconstant, even with a strain hæmolytic on blood agar. Diagnostic anti-hæmolytic sera must therefore be selected for a high content of either *œdematiens* or *septicum* antihæmolysins and a low antihæmolysin content for the other of these two species, as well as for *Cl. welchii*, *Cl. tetani* and *Cl. chauvoei*.

A serum containing moderate amounts of antihæmolysins against all five species was selected as a polyspecific standard (P.S.) and a number of horse antitoxins were tested in parallel with it, using fresh hæmolysins from overnight cultures (table I). One volume of each of a series of antiserum dilutions was mixed with one volume of hæmolytic culture and the mixtures incubated for 20 minutes at 37° C. ; two volumes of 3 per cent. washed human red cells were then added and the results read after one hour at 37° C., the tubes being shaken at approximately 20-minute intervals during incubation. Antihæmolysin titres were expressed as the reciprocals of the highest final dilutions of serum in which there was less than 50 per cent. hæmolysis. The antihæmolysins in the test antitoxins were then each specified as the ratio (R) of test antitoxin titre to the corresponding P.S. titre.

Monospecific antisera are required that will neutralise the hæmolysins likely to be present in overnight Brewer's medium cultures of any strain that turns up in medical bacteriological practice. The polyspecific standard was therefore tested against overnight cultures of a large number of representative strains of each of the five hæmolytic clostridia and the range of antihæmolysin

titres obtained (table II). The limiting values of the range of antihæmolysin titres in any antitoxin tested are then obtained simply by multiplying the limiting values of the P.S. ranges by the appropriate values of R. Many antisera thus tested were rejected, since there was no dilution at which they were monospecific. A serum suitable for monospecific neutralisation of *œdematiens* hæmolysins is illustrated in tables I and II, where there is a large

TABLE I

Titration of Cl. œdematiens *antiserum in parallel with polyspecific standard (P.S.)*

Hæmolysin	Serum	Dilutions of serum							R
		1:50	1:100	1:200	1:400	1:800	1:1600	1:3200	
Cl. œdematiens	Cl. œdematiens	−	−	−	−	−	−	+	$\frac{1600}{800} = 2$
	P.S.	−	−	−	−	−	+	+	
Cl. septicum	Cl. œdematiens	−	−	+	+	+	+	+	$\frac{100}{400} = 0 \cdot 25$
	P.S.	−	−	−	−	+	+	+	
Cl. welchii	Cl. œdematiens	−	−	+	+	+	+	+	$\frac{100}{800} = 0 \cdot 125$
	P.S.	−	−	−	−	−	+	+	
Cl. tetani	Cl. œdematiens	−	−	+	+	+	+	+	$\frac{100}{400} = 0 \cdot 25$
	P.S.	−	−	−	−	+	+	+	
Cl. chauvoei	Cl. œdematiens	−	+	+	+	+	+	+	$\frac{50}{50} = 1$
	P.S.	−	+	+	+	+	+	+	

+ = at least 50 per cent. hæmolysis
− = less than 50 per cent. hæmolysis

TABLE II

Ranges of antihæmolysin titres of polyspecific standard (P.S.) and calculation of corresponding ranges of Cl. œdematiens *antiserum*

Hæmolysin	Antihæmolysin titres of P.S. (observed)		Range of antihæmolysin titres of *Cl. œdematiens* antiserum (calculated)
	No. of strains tested	Range	
Cl. œdematiens	15	800-51,200	1600-102,400
Cl. septicum	16	50-1600	12·5-400
Cl. welchii	17	100-1600	25-200
Cl. tetani	16	400-1000	100-400
Cl. chauvoei	1	400	400

gap between the highest concentration (1 : 1600) likely to be required for neutralisation of *Cl. œdematiens* hæmolysins and the lowest (1 : 400) likely to neutralise any of the other four. It must be emphasised, however, that these two concentrations were determined by reference to strains so far tested, and although it is believed that they represent the limits of variation in hæmolysin production in Brewer's medium, the serum used within the range of 1 : 400

to 1 : 600 might fail to neutralise an exceptionally powerful homologous hæmolysin, or might neutralise an exceptionally weak heterologous hæmolysin. It follows that the larger the gap the more reliable each " monospecific " anti-hæmolysin will be.

The volume of antiserum necessary to neutralise a particular hæmolysin in the test volume depends on the ratio

$$\frac{\text{combining power of hæmolysin in test dose}}{\text{units antihæmolysin per unit volume antiserum}}.$$

If a culture contains more than one hæmolysin, that for which this ratio is greatest will be the one for which the end-point is determined. It will be seen that the validity of the method depends on the assumption that if more than one serological type of hæmolysin is produced by any of the species, the one for which the end-point is determined is always the same. If this assumption is invalid, misleading results of the type described by Oakley and Warrack (1941) would be obtained and the range of monospecific neutralisation wrongly estimated. The last possibility may be guarded against by testing the anti-serum chosen, at the dilution decided upon for the diagnostic test, against overnight cultures in Brewer's medium of as many strains as possible of the five hæmolytic species. It should consistently neutralise all homologous and fail to neutralise all heterologous hæmolysins (see below).

As immunisation of horses for the production of therapeutic antitoxin proceeds, the antihæmolysin titres of the sera often change rapidly. For example, in a month the concentration of *œdematiens* antihæmolysin in one horse's serum fell to one-quarter of its original value, while at the same time the concentration of *septicum* antihæmolysin rose 16-fold. For this reason it is important to bleed the horses for " monospecific " antihæmolytic sera as soon as possible after suitable sera have been indicated by titration of test bleedings.

Red cells. Human and rabbit cells are suitable : sheep cells are not (Miss G. H. Warrack, personal communication). The test was standardised with human cells, since these are the most likely to be available in field conditions. The cells must be washed with saline to free them from serum, which may contain natural antibody. Cells from 3 human subjects behaved identically in tests of 2 sera with the hæmolysins of *Cl. œdematiens, Cl. septicum, Cl. welchii* and *Cl. tetani.*

Incubation of hæmolysin tests. During the present investigation the pre-liminary incubation period of 20 minutes at 37° C. for culture and antiserum and the incubation for one hour at 37° C. after adding the red cells have not been varied. A few tests showed that six hours' incubation of the hæmolytic mixture did not substantially alter either *septicum* or *œdematiens* titres.

The test

Reagents. (1) Eighteen- to 24-hour Brewer's medium culture of the organism to be identified ; a portion of a colony on a solid medium is a suitable inoculum for this Brewer's medium culture. Cultures used for hæmolysin tests must be pure. (2) 0·2 M sodium acetate buffer, pH 5·2. (3) Antihæmolytic sera diluted to a monospecific concentration in buffer. (4) Human red cells, washed 3 times in saline and made up as a 3 per cent. suspension in saline, the deposit after centrifuging being reckoned as 100 per cent. red cells.

Method. For each culture, 3 tubes are set up as shown in table III, a unit volume of 0·25 c.c. being suitable. If desired, each batch of tests may be controlled fully with a set of 3 tubes in which uninoculated medium replaces culture. There should be no hæmolysis in any of the control tubes.

Interpretation of readings. A *Clostridium* that produces a hæmolysin neutralised in tube 2 but not in tube 3 is *Cl. œdematiens* ; *Cl. septicum* is indicated by neutralisation in tube 3 but not in tube 2. A hæmolysin neutralised in tubes 2 and 3, or in neither tube, indicates that the organism is not *Cl. œdematiens* or *Cl. septicum* and may be *welchii, tetani, chauvoei* or *bifermentans*. Absence of hæmolysis in all three tubes may mean either that the organism does not form a hæmolysin or that it is at the wrong stage of growth for adequate hæmolysin production (see *Time relations of hæmolysin production,* p. 13).

TABLE III

Method of hæmolysin test

Tube 1	Tube 2	Tube 3
1 volume culture	1 volume culture	1 volume culture
1 volume buffer, pH 5·2	1 volume *œdematiens* anti-serum diluted in buffer, pH 5·2	1 volume *septicum* anti-serum diluted in buffer, pH 5·2

Tubes shaken and incubated for 20 minutes in water-bath at 37° C.
2 volumes 3 per cent. human red cells added to all tubes.
Tubes incubated for one hour in water at 37° C., with shaking at 20-minute intervals; then centrifuged and read for hæmolysis.

Results

The following known strains were tested with an *œdematiens* and a *septicum* monospecific serum selected by the tests described above. *Cl. œdematiens* (15) and *Cl. septicum* (16) showed clear-cut identification ; *Cl. tetani* (16), *Cl. welchii* (17 of 23), *Cl. chauvoei* (1 of 2) and *Cl. œdematiens* (*gigas*) (1 of 2) were hæmolytic for human red cells, but none gave a false positive identification of *Cl. septicum* or *Cl. œdematiens*. These results were confirmed, as was also the specificity of neutralisation by the antisera selected, by Professor McIntosh with *Cl. œdematiens* (3 of 4), *Cl. septicum* (1) and *Cl. welchii* (1 non-hæmolytic) and by Dr J. Keppie with *Cl. œdematiens* (3), *Cl. septicum* (3) and *Cl. œdematiens* (*gigas*) (4 hæmolytic). Professor McIntosh's non-hæmolytic strain of *Cl. œdematiens* yielded the specific hæmolysin in further tests.

In tests of aerobes, strains of spore-bearing bacilli (4), *Strep. pyogenes* (3), *Strep. fœcalis* (2), *Bact. coli* (2), paracolon bacilli (2), *Staph. aureus* (1), *Staph. albus* (1) and *Proteus vulgaris* (1) failed to hæmolyse under the conditions of the test ; one each of *Strep. pyogenes* and *Bact. coli* produced hæmolysins, neither of them neutralised by the two antisera ; two spore-bearing bacilli produced hæmolysins neutralised by both antisera.

During the routine examination of wound specimens the hæmolysin test was applied for diagnostic purposes to 181 overnight cultures of anaerobes, of which 24 were tested independently by two colleagues.

The results are summarised in table IV. The atypical *Cl. œdematiens* includes strains that fermented lactose or sucrose, failed to liquefy

TABLE IV

Routine tests on 181 overnight pure cultures

Antihæmolysin	Results of test				
Nil . . . . Cl. œdematiens . . . . Cl. septicum	+ − +	+ + −	+ + +	+ − −	− − −
Cl. œdematiens (typical) . .	10				1
Cl. œdematiens (atypical) . .	8			2	10
Cl. septicum		11			1
Cl.·welchii			17		2
Cl. tetani			14		4
Cl. bifermentans			3	4	12
Cl. capitovale				1	2
Cl. putrificum				1	1
Cl. sporogenes					32
Unidentified clostridia (neither Cl. septicum nor Cl. œdematiens)			4	11	21
Other anaerobes * . . .					9

* Including *Cl. sphenoides* (2) and one strain each of *Cl. histolyticum, Cl. tetanomorphum, Cl. butyricum, Cl. cochlearium, Cl. hastiforme, fusiformis* and an anaerobic coccus.

Percentage of *Cl. œdematiens* missed 42
Percentage of *Cl. septicum* missed 8
Percentage " *œdematiens* " reactions not *Cl. œdematiens* . . . 0
Percentage " *septicum* " reactions not *Cl. septicum* 0

gelatin or failed to ferment maltose, but resembled typical *Cl. œdematiens* in all other morphological, cultural and biochemical characters. Of these 20 strains, 12 produced gas gangrene in mice and were considered to be more closely related to *Cl. œdematiens* than to any other Clostridium, although their identity is still under investigation. Animal protection tests have not been possible because the available sera when diluted to a point beyond that at which heterologous antitoxins are active, have been too weak to yield clear-cut answers with minimal numbers of animals. It will be seen (table IV) that 13 (42 per cent.) of the 31 strains which subsequently proved to be *Cl. œdematiens*, either typical or atypical, were not identified by the test, but it is interesting to note that fairly extensive examination failed to identify precisely an even higher proportion, since 20 of them (65 per cent.) were atypical. The hæmolysins of the atypical strains may have been neutralised by unrecognised heterologous antibodies in the *Cl. œdematiens* antihæmolysin.

The hæmolysin test has been used successfully under field conditions by Major MacLehnan in the examination of 50 strains of anaerobes. He found the test simple enough to be practicable with restricted facilities. It is in addition reasonably reliable and yields results within a short time of the receipt of specimens.

Discussion

Antiserum-controlled haemolysin tests are recommended for the identification of *Cl. œdematiens* (excluding *gigas* strains) and *Cl. septicum* because they are easy to apply and yield results quickly. With selected antisera, positive results are fully reliable with both *Cl. septicum* and *Cl. œdematiens*. Negative results are infrequent with *Cl. septicum* and typical *Cl. œdematiens*, though frequent with atypical *Cl. œdematiens*. Results are obtained usually within 48 hours of the initial plating of wound material.

Preliminary examination of the cultures will yield two useful contra-indications for the haemolysin test, namely the formation of terminal spores and the early production of a foul odour in cooked meat medium, either of which is conclusive proof that an unknown pure culture is neither *Cl. œdematiens* nor *Cl. septicum*.

It must be emphasised that the production of a serologically specific haemolysin by these two organisms does not indicate with certainty that the strain is toxigenic, as, for example, the Nagler reaction does in the case of *Cl. welchii*. The haemolysins tested are not necessarily the only haemolysins that the organisms are capable of producing. With *Cl. œdematiens* the activity is almost certainly not that of the lethal toxin (Dr C. L. Oakley and Miss G. H. Warrack, personal communication). The relation of the haemolysin found in Brewer's medium cultures of *Cl. septicum* to the lethal toxin is at present unknown (but see Bernheimer, 1944).

Summary

In Brewer's medium *Cl. œdematiens* and *Cl. septicum* form haemolysins for human red cells which are species-specific and can be neutralised by selected antihaemolytic sera. To allow the maximum activity of *septicum* haemolysin, test mixtures must be buffered to a *p*H of about 6·0. Serologically controlled tests for these haemolysins permit the identification of *Cl. œdematiens* and *Cl. septicum* within 48 hours of plating wound material. A standardised test made on human red cell suspensions with cultures in Brewer's medium at *p*H 6·0, controlled by selected monospecific antihaemolytic horse sera, has proved satisfactory for this purpose.

Other anaerobes, chiefly *Cl. welchii*, *Cl. tetani*, *Cl. chauvoei* and *Cl. œdematiens* (*gigas*),—and some aerobes are haemolytic under the conditions of the test but their haemolysins are either neutralised by both or not neutralised by either of the two antisera. *Cl. bifermentans*, *Cl. capitovale* and *Cl. putrificum* occasionally form a haemolysin; *Cl. histolyticum*, *Cl. sporogenes*, *Cl. hastiforme* and *Cl. sphenoides* are not haemolytic under these conditions.

The test is easy to apply and provides a reliable means of identification for *Cl. septicum* and for typical *Cl. œdematiens*. Atypical strains of *Cl. œdematiens* tend to give false negative results.

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NOTES ON THE WEIL-FELIX REACTION IN TYPHUS FEVER AND OTHER DISEASES

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This article presents the results obtained with the Weil-Felix reaction in cases of pyrexia admitted to a large General Hospital in the Middle East between March 1942 and February 1944. These included over 4500 cases of dysentery, 1500 of malaria, 200 of enteric group fevers and over 5000 others, mostly short-term fevers. Eighty cases of typhus were studied, the largest numbers of cases per month being 10 and 9 in March and April 1943 respectively : the clinical aspects of most of these cases have been reported (Crofton and Dick, 1944). The Weil-Felix reaction was usually tested on the 3rd or 4th day after admission, with the appearance of the rash, *i.e.* about the 5th or 6th day of the disease. In every case malaria was eliminated by examination of blood films and septic infection by a white cell count. The value of the latter in the differential diagnosis of typhus and the enteric group has been discredited by Crofton and Dick. Blood culture for the enteric group of organisms was performed at the same time as the Weil-Felix test. If the diagnosis of typhus was probable or if the pyrexia was maintained and its origin still undetermined, the Weil-Felix reaction was repeated about the 10th to the 12th day of illness and again later if necessary.

During the period under review, 505 Weil-Felix reactions were performed on 308 cases, comprising 80 cases of typhus, 62 of enteric group fevers and 166 cases of various other fevers. The results are recorded here to show that the reaction was a highly specific test for typhus fever, provided the observer was aware of its limitations and fully acquainted with its interpretation.

METHOD

The patient's serum was put up in a series of doubling dilutions in normal saline from 1 : 30 to 1 : 1920 in Dreyer's agglutination tubes against the standard R.A.M.C. suspensions of *B. proteus OX* 19, *OX* 2 and *OX* K and left overnight in the 37° C. incubator. The results were read next morning in strong light (daylight in Egypt was most satisfactory) against a dark background. The end-point taken was the last tube in which a definite ring of particles could be obtained by rotation, and seen with the naked eye, at the neck of the tube : usually this was one tube (occasionally two) beyond that in which complete

agglutination occurred. Thus the readings given below are probably higher than would have been obtained after 2 or 4 hours at 37° C. and leaving overnight in the refrigerator, but lower than if the test had been read after 2 or 4 hours at 50° C.

RESULTS

Agglutination with *B. proteus OX* 19 and *OX* 2 suspensions to titres of 1 : 30 and 1 : 60 was observed in several control sera (negative Kahn sera from afebrile patients) and in many of the cases, and was considered to be of no significance. Titres of 1 : 120 and 1 : 240 were found occasionally in cases of pyrexia which eventually proved to be other than typhus ; in such instances the specificity of the antigen employed was checked against known typhus and non-typhus sera. Such non-specific reactions were of more frequent occurrence with *OX* 2 than with *OX* 19 suspensions.

Within the first 10 days of a patient's illness, a titre of 1 : 240 was reported as " slightly suggestive " and the test repeated after the 10th day : every case which showed a rise in titre above this proved to be clinical typhus. A titre of 1 : 480 before the 10th day was considered " very suggestive " and repetition usually showed a definite rise in typhus patients and a fall in others. Titres over 1 : 480 were regarded as definite positives, with the single exception of an unexplained high reading in a case of paratyphoid B fever.

With *B. proteus OX* K suspensions, non-specific reactions up to 1 : 480 were relatively common and experience showed that even 1 : 960 could not be accepted as definitely positive. As a rule, in cases positive clinically and in tests with *OX* 19 and *OX* 2, titres with *OX* K did not rise above 1 : 240. There were no indications that scrub (mite-borne) typhus was present in the Suez Canal zone of Egypt. Accordingly the use of *OX* K suspensions was discontinued after a year's thorough trial, a policy adopted by others, including van Rooyen and Bearcroft (1943).

(i) *The Weil-Felix reaction in typhus fever*

The 80 cases in this group represented all degrees of clinical severity and, while the clinician could make a reasonably assured diagnosis in the severe and typical cases, the laboratory findings were of great importance in the recognition of mild and atypical cases. The Weil-Felix reaction was the only practicable laboratory test available on the spot. Subsequently Major C. E. van Rooyen, working in collaboration with Dr James Craigie of Toronto University, performed rickettsial agglutinations on sera from 68 of these cases and showed that 21 were of epidemic (*i.e.* louse-borne) and 36 of murine (*i.e.* flea-borne) type ; in the remaining 11 the agglutinins were insufficiently developed to permit separation of these two infections by serological methods.

The murine cases were distributed throughout the two years, though mostly in autumn and among units stationed in desert camps having no contact with natives. Usually, the patients stated that rats were present in the camp or even that their tents were infested. Moreover, during the late autumn of 1943, bubonic plague made its appearance in this same area. The epidemic cases on the other hand occurred chiefly in the spring and summer of 1943, in units where large native labour corps were employed or the patient gave a history of close contact with natives. One patient, for example, worked at a Railway Transport Office in a station with natives brushing past him continually. There was a large outbreak of typhus in the native population at this time (Kamal and Messih, 1943). Very few patients were lousy on admission.

In the 80 typhus cases 147 Weil-Felix reactions were performed. Table I shows the results of these tests in relation to the

TABLE I

The Weil-Felix reaction in typhus fever. Distribution according to day of disease of 147 reactions in 80 cases

Day of disease	3	4	5	6	7	8	9	10	11	12	13	14	15	later	Total.
Negative	1	4	8	13	5	5	9	5	0	1	0	1	0	0	52
Suggestive	0	0	0	1	2	2	1	0	0	3	2	0	1	6	18*
Positive	0	0	0	1	3	4	2	9	6	8	8	5	9	22	77†
														Total =	147

Suggestive = 1 : 480.

* Highest attained in 7 cases.
† In 73 cases.

duration of the illness; titres higher than 1 : 480 were regarded as positive and in practice this criterion worked out very satisfactorily throughout the two years. Certain important points emerge from this table.

The earliest definite positive was on the 6th day of disease (see also table II, case 1). Less than one-third of the cases gave a positive or suggestive result up to and including the 10th day, although 75 sera were examined in this period of the disease. Thus a Weil-Felix reaction positive to a titre of 1 : 480 or over was not expected before the 10th day in the majority of cases and, conversely, a negative reaction at this stage was by no means exclusive of typhus. Of the two cases giving negative reactions after the 10th day, one was positive on the 17th day (table II, case 2) and the other gave a suggestive result on the 16th day, followed by a positive on the 23rd day (table II, case 3). The change from negative to positive may take place very rapidly (table II, cases 4 and 5). In seven cases, a titre of 1 : 480 was the highest obtained (e.g. table II, case 8), though the diagnosis was definite clinically; three of these were epidemic and two murine by rickettsial agglutination.

TABLE II—*Examples of Weil-Felix reactions referred to in text*

Case	Day of disease	Titre with			Remarks
		OX 19	OX 2	OX K	
A. Typhus fever					
1	6	1920+	960	...	Earliest definite positive
2	9	60	60		Slow to become positive and only with OX 2 : rat catcher at Infantry Training Depot
	14	120	240	...	
	18	30	960		
3	5	0	30		Slow to become positive : anti-typhus inoculations six weeks before onset of typhus
	12	240	240		
	16	480	480	...	
	23	960	120		
4	4	120	0		Rapid change from negative to positive
	8	1920+	120	...	
5	7	240	240	...	Rapid change from negative to positive
	10	1920+	120	...	Serum obtained *post mortem*
6	6	0	120		Positive only with OX 2 : anti-typhus inoculations eight weeks before onset of typhus
	13	60	960	...	
	21	120	480		
7	10	120	120		OX 2 agglutination early : OX 19 agglutination late (in convalescence)
	16	120	1920	...	
	34	1920	240		
8	15	480	240		OX 19 agglutination early
	21	240	480	...	OX 2 agglutination late
9	8	1920+	120		Fall in titre during convalescence
	24	480	120	...	
B. Other diseases					
10	6	30	120		Typhoid fever : toxic, delirious : no rise in titre
	11	30	120	...	
	22	60	120		
11	12	30	240		Typhoid fever, late in coming under observation : relatively mild : fall in titre during course of disease
	17	0	60	...	
	21	30	60		
12	4	240	240		Paratyphoid A fever : toxic
	12	240	60	...	
	22	120	120		
13	14	960	960		Paratyphoid B fever : severe; (?) double infection
	16	960	960	...	
	90	60	30		
14	3	120	60		Influenza
	8	30	60	...	
15	5	120	120		Gastritis
	11	0	0	...	
16	10	60	240		Glandular fever with rash like typhus : anti-typhus inoculations four weeks before onset of fever
	14	60	240	...	
	23	60	120		
17	4	120	30		Pyrexia of unknown origin with atypical rash
	12	120	120	...	
	19	60	120		
18	6	30	30		Tonsillitis with atypical rash : developed diphtheria on 16th day—during convalescence—and shows slight rise in titre with the intercurrent infection
	14	120	120		
	23	240	240	...	
	32	120	120		
19	3	0	30	...	Dysentery, indefinite exudate
	8	120	60	...	Now *convalescent*
				OX K	
20	13	0	0	480	A case of lymphadenoma, fatal three months after the first symptoms
	20	0	0	960	
	27	60	240	960	
	43	30	240	960	
	64	60	120	480	
	70	120	240	240	

The times at which a positive Weil-Felix reaction developed in the murine and epidemic types of typhus were compared and it appeared that a positive titre occurred on the average at a slightly later stage of the disease in the murine cases.

No attempt was made to determine the titre when it was beyond 1 : 1920, nor to discover how long after the illness the high titre persisted, though a few cases gave some information on the latter point. In two cases (table II, cases 6 and 9), a fall in titre had occurred by the 21st and 24th days, but in three others (*e.g.* case 7) the titre was still 1 : 1920 on the 27th, 34th and 36th days of illness respectively.

The titre with *B. proteus OX* 19 was usually higher than with *B. proteus OX* 2 suspension. In seven, however, a positive titre was obtained only with the *OX* 2 (*e.g.* table II, cases 2 and 6). These cases, one epidemic and six murine, could not be distinguished clinically from those positive with *OX* 19. Thus in dealing with murine or mixed epidemic and murine infections the importance of including the *OX* 2 suspension was obvious. The *OX* 2 agglutination, however, was by no means specific for the murine cases, the majority giving higher titres with *OX* 19. Cases of typhus giving higher titres with *OX* 2 than with *OX* 19 have also been reported by van Rooyen and Bearcroft (1943), Baker *et al.* (1943), Brockbank and Whittaker (1944), van Rooyen *et al.* (1944) and van Rooyen (1944).

Megaw (1945) has raised the question of the possible existence of tick typhus in Egypt. Both the author and van Rooyen have been on the alert for possible cases and, although blood was inoculated into guinea-pigs from high titre *OX* 2 cases, only epidemic virus was obtained. Likewise a few efforts were made to isolate rickettsiae from *R. sanguineus* gathered in Egypt, but without success. There was no evidence, clinical or bacteriological, to warrant the claim that the cases of *OX* 2 agglutination studied were due to tick-borne infection.

(ii) *The Weil-Felix reaction in enteric group fevers*

In 62 cases of enteric fevers, 90 Weil-Felix reactions were carried out at various stages of the disease. The sera from 9 out of 26 cases of typhoid fever gave titres of 1 : 120 with *B. proteus OX* 19, *OX* 2 or both, three of them reaching 1 : 240 with *OX* 2; in most of these agglutination was not complete and there was no rise in titre on repetition (*e.g.* table II, case 10). One medical officer who had received anti-typhus inoculations six months and one year before gave titres of 1 : 120 with both *OX* 19 and *OX* 2 on the 7th day of typhoid fever. In the three cases in which titres of 1 : 140 with *OX* 2 were reached on the 4th, 9th and 12th day respectively, the agglutination in the 1 : 120 dilution was "total" and the diagnosis of typhus was necessarily considered. The case with this titre on the 4th day had been in close contact with another case of typhoid fever in circumstances in which typhus could be excluded. That on the 9th day gave no rise of titre on repetition and was clinically typhoid. The third case (table II, case 11) showed a definite fall

in titre five days later and gave no other indications of typhus : this seemed to indicate that a non-specific rise in agglutinins to *OX* 2 had occurred in the early stages of typhoid fever, as distinct from the "normal" agglutinins of the earlier cases, whose titre remained almost constant (*cf.* Felix, 1943-44).

In tests on 28 cases of fevers due to *Bact. paratyphosum A* or *B*, the sera from eight reached a titre of 1 : 240 with *B. proteus OX* 19, *OX* 2 or both, and in six of them agglutination was complete up to the dilution of 1 : 120 : on repetition, however, the titre did not rise and the subsequent clinical course and results of blood cultures showed the true diagnosis. In two cases in which the rash was not typical of enteric, early torpor was present and an early Weil-Felix reaction gave a "slightly suggestive" result ; the failure of the titre to rise on repetition was very helpful (table II, case 12). Case 13 was admitted on the 13th day from a distant area and on the results of blood culture and first Weil-Felix reaction was considered to be a possible double infection ; as against this, no other cases of typhus had occurred in that area, two other cases of paratyphoid B had been admitted from the camp and clinically there were no other indications of typhus. With serum taken on the 16th day, Major van Rooyen found no agglutination with epidemic and only to 1 : 50 with murine rickettsiæ. In two cases of paratyphoid A fever, titres of 1 : 240 with *OX* 19 and 1 : 120 with *OX* 2 were obtained on the 5th and 8th days and these had fallen to 1 : 30 with both suspensions by the 27th and 32nd days respectively.

(iii) *The Weil-Felix reaction in other fevers*

In 166 cases of fevers other than typhus and the enteric group 268 Weil-Felix reactions were performed, some early in the disease, others after the 10th day, with the results shown in table III.

TABLE III

Results of Weil-Felix reactions in 166 fevers other than typhus or the enteric group

		Titres					
		Negative	1:30-1:60	1:120	1:240	1:480	Totals
OX 19	Up to 10th day	78	70	26	2	0	176
	11th day and after	38	40	10	4	0	92
	Total	116	110	36	6	0	268
OX 2	Up to 10th day	38	96	35	5	2	176
	11th day and after	23	31	27	11	0	92
	Total	61	127	62	16	2	268
No. of cases showing highest titres in either		22	80	52	10	2	166

In 22 cases no agglutination was obtained with *B. proteus OX* 19 or *OX* 2 suspensions at any stage of the fever. Agglutination to maximum titres of 1 : 30 or 1 : 60 with one or other or both suspensions was obtained at some stage of the illness in 80 cases : not being " total ", these were considered to be of no significance. The diseases sampled in these groups included malaria (benign and malignant tertian), sand-fly fever, acute miliary tuberculosis, meningitis (meningococcal and benign lymphocytic, both with rashes), diphtheria with acute rheumatic fever, diphtheria with serum rash, amoebiasis of colon and liver, kala-azar and plague.

The conditions which gave rise to titres of 1 : 120 and over were of greater interest. These non-specific agglutinations were not constant for these diseases and occurred more frequently with *OX* 2 than with *OX* 19 suspensions. In most cases the high titre was obtained early and fell later in the disease (*e.g* table II, cases 14 and 15) : in others it was fairly constant throughout (*e.g.* cases 16 and 17), while in a few the rise occurred at a late stage (*e.g.* cases 18 and 19).

(*a*) *Measles.* The diagnosis was established clinically before the Weil-Felix reaction could be of use, but an early non-specific rise in titre might temporarily have confused the issue. Three out of six cases tested showed a titre of 1 : 120 with *OX* 19, *OX* 2 or both and a fourth gave 1 : 480 with *OX* 2 up to the 7th day, but later tests gave lower titres.

(*b*) Various *septic conditions* gave reactions as follows :—

Tonsillitis, with irregular streptococcal rash (table II, case 18)	1 : 240 (*OX* 19 and *OX* 2)
Bacterial endocarditis	1 : 120 (*OX* 19)
	1 : 240 (*OX* 2)
Gangrenous appendicitis with suppurative pyle-phlebitis	1 : 120 (*OX* 2)
Cellulitis of foot	1 : 120 (*OX* 19 and *OX* 2)

(*c*) *Respiratory tract infections* occasionally caused some rise in titre of the Weil-Felix reaction. One case of common cold gave a titre of 1 : 480 (*OX* 2), and two cases of influenzal bronchopneumonia in Indians reached 1 : 240 (*OX* 2). Cases of lobar pneumonia, bronchopneumonia, influenza and acute bronchitis produced titres of 1 : 120 with *OX* 19, *OX* 2 or both (*e.g.* table II, case 14).

(*d*) *Infective hepatitis.* In two out of eight cases titres of 1 : 120 (*OX* 19 and *OX* 2) were obtained in the later stages of illness.

(*e*) *Glandular fever.* A few cases classified under this heading, especially those with rashes, gave rise to suspicion of their being typhus when the blood changes were not typical. Two in this series gave titres of 1 : 120 with *OX* 2 on the 8th and 11th days respectively. A third (table II, case 16) gave a titre of 1 : 240 ; this patient had been given anti-typhus inoculations one month previously. In no case did the titre rise higher.

(*f*) *Bacillary dysentery* and *gastro-enteritis* each provided two cases with titres of 1 : 120, in the former at a late stage when there was no doubt as to the diagnosis (*e.g.* table II, case 19), but early in the latter and the subsequent fall in titre was very helpful (*e.g.* table II, case 15).

(*g*) *Pyrexia of unknown origin.* In several cases of pyrexia whose origin remained undetermined, repeated Weil-Felix reactions were performed (*e.g.* table II, case 17) ; the failure of the titre to rise over 1 : 240 throughout the

illness and convalescence was considered to exclude a diagnosis of atypical typhus.

(h) *Lymphadenoma*. In this case (table II, case 20) agglutination with *B. proteus* OX K suspension caused considerable confusion. The patient ran a low temperature like a mild enteric fever, with sweating, headache, backache and diarrhœa for eight days ; no rash was noticed. There followed an evening rise of temperature to 100° F. for two weeks, with the spleen just palpable, and thereafter a swinging temperature between 100° and 102° F. lasted for several weeks. The Weil-Felix reaction was performed at the times shown, and was continued after the diagnosis of lymphadenoma had been established on account of the agglutination titres reached with OX 19 and OX 2 as well as with OX K. In the early stages the OX K titres were considered to be of possible significance, and rickettsial agglutinations with samples of serum yielded the following results.

Day of Illness	Rickettsiæ	
	Epidemic	Murine
13th	0	500
27th	0	500
64th	0	200

Thus this case of lymphadenoma produced a definite and confusing rise in the Weil-Felix titre, accompanied by low titre non-specific rickettsial agglutination.

The effect of anti-typhus inoculation on the Weil-Felix reaction

Seven of the cases in this series had received anti-typhus inoculations. Two of these (table II, cases 3 and 6) developed typhus fever of murine type six and eight weeks after the inoculations.

The results in these cases do not materially assist the arguments either of Süpfle and Fischer (1943), that the inoculation caused a lower titre in a subsequent attack of typhus, or of Ding (quoted by Felix), who maintained that the reaction was not affected. They do, however, confirm Felix's point that a rising titre is obtained in the inoculated as in the uninoculated subject. In the other five cases the previous inoculation failed to affect the results of the reaction, except possibly in case 16 (table II) where the titre may have been slightly elevated. Penfold (1944) also reported that following inoculation and reinoculation with Cox vaccine the highest OX 19 agglutinin titre reached only one of 1 : 160.

DISCUSSION

The great majority of the personnel in the units from which these patients came were British ; the remainder were Indians, South Africans and other races among whom typhus is not endemic. Thus it was expected that titres for normal persons and in diseases other than typhus would have conformed to the findings published by the Medical Research Council (1942) and many other workers, *e.g.* Mackenzie (1941-42), Maly (1942), McConn (1943) and Felix (1943-44), who gave the diagnostic titre for typhus fever in non-endemic areas

as 1 : 100 or at most 1 : 200 with *B. proteus OX* 19. All patients in this series had been fully inoculated with T.A.B. vaccine and tetanus toxoid. These inoculations appear to have had some effect in activating the patient's "shadow factories" for manufacturing agglutinins, and occasionally various febrile conditions produced a slight nonspecific rise in the Weil-Felix titre. This rise was not nearly so marked as the anamnestic rise in the Widal reaction, which is now recognised to be of little value in the diagnosis of enteric fevers in previously vaccinated patients (Boyd, 1942 ; Süpfle and Fischer, 1943 ; Felix, 1944).

There was no epidemic of typhus during the period of investigation. In the wide range of diseases studied, the number of cases which produced titres of 1′: 120 (58 cases) and 1 : 240 (21 cases) and which proved not to be typhus was considerable: even a titre of 1 : 480 was not always diagnostic. On the other hand, no case clinically like typhus failed to give this last titre with either *OX* 19 or *OX* 2 suspensions. The value of the Weil-Felix reaction as an early aid to diagnosis has been pointed out by Findlay (1941-42) and Felix (1943-44). The occurrence of low titre curves (1 : 50 after a previous negative) and the significance of a 100 per cent. rise at low levels has been emphasised by Felix. We have not, however, felt justified in considering titres below 1 : 480 as diagnostic and other workers have adopted a similar policy in respect of titres below 1 : 200 or 1 : 400 (Maly, 1942 ; van den Ende *et al.*, 1943 ; Robinson, 1943 ; Baker *et al.*, 1943 ; Kamal and Messih, 1943 ; Brookbank and Whittaker, 1944 ; Sancho Lobo, 1944). Care should be exercised in the interpretation of a rising titre at low levels because of the possibility of a non-specific rise in agglutinins for *OX* 2 in typhoid and for both *OX* 19 and *OX* 2 in paratyphoid fevers ; as noted above, other conditions may produce small rises in titre either early or late in the disease. The necessity for caution has already been pointed out in cases of chronic brucellosis (Calder, 1941) and in patients who have had Proteus group infections (Medical Research Council, 1942 ; Dammin and Billings, 1942 ; Sonnenschein, 1943). The rise in titre in the case of lymphadenoma is interesting in view of the results obtained by Grateh (1943) in pregnancy and malignant disease.

SUMMARY

1. The results of 505 Weil-Felix reactions in 308 cases of pyrexia, including 80 of typhus fever, admitted to a large military hospital in Egypt are reviewed.

2. A Weil-Felix reaction positive to a titre of 1 : 480 with *B. proteus OX* 19 or over 1 : 480 with *B. proteus OX* 2, using the technique described, was a highly specific diagnostic test for typhus fever as met with in this area, in military personnel.

3. Titres of 1 : 480 or over were obtained only after the 10th

day of illness in the majority of cases of typhus : lower reactions before this day did not exclude the possibility of typhus.

4. Titres up to 1 : 240 and occasionally to 1 : 480 occurred as non-specific reactions in enteric and various other febrile conditions, such as measles, septic lesions, respiratory tract infections, infective hepatitis, glandular fever and dysentery.

5. It is suggested that the non-specific reactions might be due to previous inoculation with T.A.B. vaccine and tetanus toxoid.

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576 . 851 . 51 (*pleuropneumonia*) : 616 . 64 + 618 . 1

THE ISOLATION OF ORGANISMS OF THE PLEURO-PNEUMONIA GROUP FROM THE GENITAL TRACT OF MEN AND WOMEN

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(PLATES I AND II)

THERE have been reports in recent years of the isolation of organisms of the pleuropneumonia group (L organisms) from the genital tract of men and women, and it has been suggested that they may be the cause of so-called non-specific urethritis and cervicitis, conditions from which no known human pathogen has hitherto been isolated. The fact that several members of this group produce arthritis in animals makes this an attractive hypothesis, since arthritis is as common a complication of " non-specific " genital infections in man as it is of gonorrhœa. Moreover, the fact that L organisms cannot be recognised in ordinary smears and are difficult to cultivate may explain why they have been missed in the past.

Dienes (1940) and Dienes and Smith (1942) in America have published reports on the isolation of L organisms from the cervical, vaginal and urethral secretions of women and the urethral and prostatic secretions of men. Some of the women were suffering from gonorrhœa, some from non-specific inflammations and some from arthritis ; others were clinically normal All the men, however, showed signs of chronic prostatitis. Beveridge (1943), in Australia, has confirmed the occurrence of L organisms in men. He found them in 4 out of 24 non-specific urethral discharges. Klieneberger-Nobel (1945), working at the Whitechapel Clinic and the London Hospital, records the isolation of L organisms from the vaginal secretions of 40 per cent. of women attending the venereal diseases clinic, 33 per cent. of women attending the gynæcological department suffering from various presumed non-venereal conditions, but only 14 per cent. of women attending the antenatal clinic.

The present study formed part of an investigation of non-specific urethritis and cervicitis. Among soldiers in the large area served by this hospital, out of every three men who report sick with a urethral discharge, one has non-specific and two have gonococcal urethritis Among women in the Services the proportion of non-

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specific genital inflammation is apparently higher, partly perhaps because of technical difficulties in the isolation of the gonococcus from women. Similar figures have been noted in other areas. Since the non-specific cases are, in general, more resistant to treatment than the gonococcal and at least as liable to serious complications, it will be realised that they constitute a serious problem. We shall describe only that part of the investigation which consisted in the attempt to isolate L organisms from these patients.

Methods

After a number of special media had been tried it was found that L organisms could be grown quite simply on ordinary 10 per cent. chocolate agar, as used for growing the gonococcus. No special additions or adjustment of the pH were found necessary. Routine cultures from women of cervical and urethral secretions and from men of urethral discharges, urine and prostato-vesicular fluids were incubated for forty-eight hours in an atmosphere of increased CO_2, and were treated with the oxidase reagent for detection of gonococcal colonies in the usual way. After this, when the plates were quite dry, pieces of medium bearing possible L colonies were cut out and placed face downwards on slides. These were flooded with Bouin's fluid and put into a moist chamber in the incubator overnight. Next day the agar was peeled off, when the colonies remained adherent to the slides. These were put into 75 per cent. alcohol for half-an-hour, rinsed in distilled water, stained in 4 per cent. Giemsa for four hours and lightly differentiated and dehydrated in graded acetone-xylol mixtures as follows.

(1) Acetone containing 2·5 per cent. xylol . 10 secs. (up to 30 secs. for gonococcal or mixed L and gonococcal colonies)
(2) Acetone 2 parts, xylol 1 part . . 30 secs.
(3) Acetone 1 part, xylol 2 parts . . 1 min.
(4) Xylol . . . . . . 10 mins. or longer

To get the best results it is necessary to control under the microscope, in xylol. If further differentiation is needed the slides are taken down the series of mixtures and up again. Preparations containing gonococcal colonies should always be controlled and differentiation continued until the peripheral zone of the gonococcal colonies becomes transparent. For routine non-gonococcal preparations the above times may be relied upon without microscopical control. The slides are removed from the xylol, shaken free of excess and mounted at once, without being allowed to dry, in D.P.X. or similar neutral mountant.

L colonies can easily be distinguished with the $\frac{2}{3}$ inch objective. This method of fixing and staining, which is one of those used by Klieneberger and Smiles (1942), with modifications by Dr C. Robinow, has proved simple and certain and can be recommended especially to those who have had no previous experience of this group of organisms.

Results

Male urethritis and prostatitis (table I). L organisms were isolated from the urethral discharge, occasionally from the urine only, of 34 per cent. of cases of gonorrhœa. They were much more rarely found in primary non-specific urethritis (7 per cent.) and in the similar condition which often follows gonorrhœa (6 per cent.). Thus our

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FIG. 1.—L colony from a culture from the cervix of a case of Trichomonas vaginitis. ×885.

FIG. 2.—Two different portions of the edge of a mixed L and gonococcal colony. Note L vesicles and darkly stained cocci   ×1200

Impression preparations fixed with Bouin's fluid through agar and stained with Giemsa

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Fig 3 — Edge of a mixed L and gonococcal colony showing network of filaments × 1200

Fig 4 — Zone of radiating filaments around a mixed L and gonococcal colony × 1200

Fig 5 — Edge of a pure gonococcal colony Note that the cocci including the large pleomorphic forms are evenly stained unlike L vesicles × 1200

Impression preparations fixed with Bouin s fluid through agar and stained with Giemsa

results offer no real evidence to suggest that non-specific urethritis is due to L infection. Nor is there evidence that L organisms are responsible for prostatitis. We have found no correlation between the presence of L infection and clinical signs of prostatitis. A larger survey is needed, however, to establish this point with certainty.

TABLE I

Isolation of organisms of the pleuropneumonia group (L organisms) from the male genital tract

	No of cases	No L-positive	Percentage positive
From urethral discharge or urinary deposit or both in different clinical groups			
Gonorrhœa	35	12	34
Non-specific urethritis	45	3	7
Residual non-specific urethritis following gonorrhœa	34	2	6
From the prostate			
Prostato-vesicular secretion	38	3	8
Signs of prostatitis	9	1	...
From "normal" men			
From urethra or urinary deposit or both .	28	4	14
Signs of genito-urinary infection (slight pyuria)	4	1	

In a group of 28 men from a skin department, none of whom had any obvious signs of urethritis, L organisms were found in the urethra or urine or both in 4. Four of the group showed slight pyuria, but only one of these was L positive. Thus it is possible, as previous work has indicated, that L organisms may exist in the urethra or elsewhere in the genito-urinary tract without causing symptoms. Here also a larger survey is needed.

Female genital infections (table II). L organisms were found in cultures from the cervix or urethra or both of 11 out of 18 cases of gonorrhœa and of 39 out of 63 cases of Trichomonas vaginitis. Where the two conditions coexisted L organisms were isolated from all of 20 cases. They were found in 8 out of 18 cases of non-specific cervicitis and in 6 out of 8 cases where this condition was complicated by Trichomonas vaginitis. In this connection it should be remembered that gonorrhœa in women is often masked, particularly when Trichomonas is present. It is possible, therefore, that in women there is an even closer association than is apparent between the gonococcus and the L organism.

Seventeen clinically normal women were examined; L organisms were isolated from only one of them. It must be noted, however, that many women after apparently successful treatment for gonorrhœa or Trichomonas vaginitis were also clinically normal, yet several of these yielded L organisms.

TABLE II

Female genital tract infections : isolation of organisms of the pleuropneumonia group (L organisms) from cervix or urethra or both

	No. of cases	No. L-positive	Percentage positive
Gonorrhœa	18	11	61
Trichomonas vaginitis	63	39	62
Non-specific cervicitis	18	8	44
Gonorrhœa and Trichomonas vaginitis . .	20	20	100
Non-specific cervicitis and Trichomonas vaginitis	8	6	75
Effects of treatment			
After treatment of gonorrhœa with penicillin or sulphathiazole	14	5	36
After treatment of Trichomonas vaginitis with local applications of Stovarsol *	68	8	12
After treatment of non-specific cervicitis with penicillin or sulphathiazole	16	6	38
Controls			
Clinically normal women	17	1	6

* Stovarsol vaginal compound (May and Baker).

Local treatment of Trichomonas vaginitis with pentavalent arsenic greatly reduces, at least for a time, the frequency of isolation of L organisms. Treatment of gonorrhœa and of non-specific cervicitis with penicillin or sulphathiazole may have some effect, but the figures are not significant. L organisms were obtained in profuse, almost pure culture from the cervix and urethra of a woman with Trichomonas vaginitis two days after the completion of a course of penicillin injections for syphilis (2·4 million units given during $7\frac{1}{2}$ days).

The frequent close association of L organisms with the gonococcus is striking. L organisms were detected in apparently pure cultures of the gonococcus by Dienes (1940) and Brown and Hayes (1942). In the present series we have found that the primary mixed cultures are of two types. In one, L colonies and gonococcal colonies grow side by side, and even when in contact preserve their individual and characteristic structure; in the other, true mixed colonies occur which are unlike those of either component organism. L vesicles and gonococci appear to be fairly evenly mixed. This is most easily seen

in the periphery of the colony (fig. 2). The growth of the L organism is more abundant than it would be when growing alone in similar conditions and the filamentous elements are generally more numerous, often extending like whiskers around the mixed colony and forming a network over its surface (figs. 3 and 4). The typical radial arrangement of L growth is obscured or absent and the vesicles tend to be smaller and more nearly circular than in pure L colonies. It is seldom hard to distinguish the vesicles from the large pleomorphic forms of the gonococcus. The latter are stained evenly though faintly by Giemsa (fig. 5), while the former appear as obvious vesicles often filled with granules (fig. 2). As is well known, there is a good deal of controversy as to the relation between L organisms and the bacteria with which they are commonly associated. We shall not speculate about the nature of the association with the gonococcus. It has been possible, in several cases, to separate the L component from mixed colonies by the use of penicillin or sulphathiazole. If a solution containing 50-100 units per c.c. of penicillin or 1 : 2000 sulphathiazole is put into a porcelain cylinder on a chocolate agar plate spread with gonococcal pus, a zone of inhibition of gonococcal growth will surround the cylinder, and in this zone pure L colonies can often be found.

Summary

Organisms of the pleuropneumonia group (L organisms) have been isolated from inflammatory exudates in the genital tract of both men and women. In both, they are commonly found in gonorrhœa (34 per cent. in men and about 60 per cent. in women) and in women they are common also in Trichomonas vaginitis and in non-specific cervicitis. They are much less commonly found in cases of the troublesome non-specific urethritis of men and are uncommon in prostatitis. They are found in a small proportion of apparently healthy men and women.

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THE INFLUENCE OF SURFACE-ACTIVE SUBSTANCES ON THE GROWTH OF ACID-FAST BACTERIA

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THE physico-chemical or bio-physical state of the medium used in the culture of bacteria has in the past received little attention. Amongst the physical properties likely to influence the growth of acid-fast bacteria that of surface tension might well be expected to be important, since these organisms normally grow as a pellicle on the surface. Surface-active substances might conceivably affect the growth in two ways, either by increasing the wetting of the bacterium by the medium and thus altering its environment, or by adsorption upon or into the bacterial envelope, thus influencing certain nutritive or growth processes. Since growth as a surface pellicle is often extremely slow with some types of acid-fast bacteria, it was thought that such alterations might in some cases lead to increased growth, either upon the surface, or in bulk if the organisms were displaced from the surface by the action of the wetting agent.

Only a few papers bearing directly on this problem and with but few surface tension depressants appear in the literature (Larson, Cantwell and Hartzell, 1919 ; Larson, 1921-22 ; Larson and Montank, 1922-23 ; Cooper, 1930), although a good deal has been done with other types of bacteria (*e.g.* Lominski and Lendrum, 1942). We have examined the effect of a number of surface-active compounds of various chemical types on three strains of acid-fast bacteria, and it appears that the chemical nature of the compound is of minor importance in comparison with its effect upon surface properties as measured by surface tension. In general, as the surface tension is progressively reduced below about 40 dynes/cm. bacterial growth is increasingly inhibited, until below about 28-30 dynes/cm. no growth at all is observed.

Methods and material

Bacteria. The strains of acid-fast bacteria used were :—(1) *Mycobacterium phlei*, no. 54 of the National Collection of type cultures ; (2) *Mycobacterium tuberculosis*, human type " DT " ; (3) *Mycobacterium tuberculosis*, avian type " 7169 ". The last two were both kindly supplied by the Veterinary Laboratory, Ministry of Agriculture and Fisheries, Weybridge.

Surface active compounds. Only neutral and anionic types were examined in view of the well known toxicity of those of the cationic type : (1) aerosol I.B. (di-butyl sodium sulpho-succinate), (2) aerosol A.Y. (di-amyl sodium sulpho-succinate), (3) amyl alcohol, (4) octyl alcohol, (5) glycerol mono-oleate, (6) lecithin, (7) potassium oleate, (water content *ca.* 15 per cent.), (8) potassium stearate, (9) sodium oleate, (10) sodium stearate, (11) sodium ricinoleate, (water content *ca.* 10 per cent.), (12) sodium lauryl sulphate (sulphonated lauryl alcohol), (13) sodium oleyl sulphate (sulphonated oleyl alcohol, water content *ca.* 30 per cent.), (14) triethanolamine lauryl sulphate (sulphonated lauryl alcohol neutralised by triethanolamine, water content *ca.* 50 per cent.). Of these, compounds (1) and (2) were pure products kindly given by Cyanamid Products Ltd., compounds (5)-(14) inclusive were commercial products kindly given by Messrs Boake, Roberts Ltd. The alcohols were ordinary laboratory samples. In the percentage concentrations referred to in the text no allowance has been made either for water content or for impurities present, but the approximate values for the former are given above.

Media. Unless otherwise stated B.A.I. (Bureau of Animal Industry of the U.S.A.) synthetic medium was used, in order to eliminate as far as possible complications arising from surface-active materials present in broth and similar media. Its composition was as follows :—

Asparagine	.	.	.	.	.	.	14 g.
Dipotassium phosphate	.	.	.	.	.	1·8 „
Sodium citrate	.	.	.	.	.	.	0·9 „
Magnesium sulphate	.	.	.	.	.	1·5 „
Ferric citrate	.	.	.	.	.	.	0·3 „
Dextrose	.	.	.	.	.	.	10·0 „
Glycerol	.	.	.	.	.	.	100·0 „
Distilled water to	.	.	.	.	.	.	1000 ml.
pH	.	.	.	.	.	.	.	6·8-7·0 (no adjustment)

The surface tension of a freshly cleaned surface of this medium was about 73 dynes/cm., *i.e.* close to that of water, but fell slowly on standing to about 54 dynes/cm., probably due to traces of surface-active impurities. This medium was also used when solidified by the addition of 2 per cent. agar ; glycerol broth and Besredka egg-yolk medium were tested for comparison.

Surface tension determination. In general the ring method, using a platinum ring and torsion balance, was employed, absolute values of surface tension being obtained by applying the correcting factors of Harkins and Jordan (1930). The surface tension was usually measured at room temperature (17-20° C.), except in the case of solid media containing agar, where measurement was made at 50° C. ; at this temperature such media are sufficiently mobile for measurement.

In a number of instances, particularly when using media containing agar which were more difficult to determine by the ring method, the Sugden modification of the maximum bubble pressure was used (see Adam, 1941). With few exceptions the values obtained by these two rather different methods agreed quite closely.

Inoculation of media. The media containing the various depressants and control media without added depressants were inoculated with a small piece (1-2 mg.) of pellicle of a young culture on glycerol broth. In some cases, particularly when the surface tension was very low so that the bacteria readily sank, the inoculum was placed on the surface of a sterile cork disc of about 10 mm. diameter. The inoculated media were then incubated at 37° C. and the results recorded at intervals depending upon the rapidity of growth. The final examination was made after 14 days with *Myco. phlei* and *Myco. tuberculosis*, avian type, and after six weeks with *Myco. tuberculosis*, human type.

Results

These are set out in the table and it is evident that chemical differences between the depressants used are quite subordinate to their surface activities as measured by surface tension With the

TABLE

Surface active substance	Concentration of depressant (per cent)	Surface tension (dynes/cm)	Liquid medium (B.A I)			Solid medium (B A I +2 per cent agar)			Surface tension at 50° C (dynes/cm)
			Myco tuberculosis, avian type	Myco tuberculosis, human type	Myco phlei	Myco tuberculosis avian type	Myco tuberculosis, human type	Myco phlei	
Octyl alcohol	0 1	45 7	+++	+	+++	++	+	+++	
Amyl alcohol	0 5	40 8	++	−	+++	+++	+	+++	
" "	0 2	44 0	+++	+	+++	++	+	+++	
Aerosol A"Y"	0 5	42 6	+++	+	+++	++	+	+++	
" "	0 1	41 5	++	−	+++	++	+	+++	
Aerosol I B	0 5	34 8	−	−	+	+	−	+	
" "	0 1	40 3	+++	−	+++	++	++	+++	42 7
Sodium stearate	0 2	37 0	++	−	+++	++	++	+++	41 1
" "	0 1	32 3	++	−	+++	++	+	+++	
" "	0 2	32 3	++	−	+++	++	+	+++	
Sodium ricinoleate	0 5	32 3	++	−	+++	++		++	
" "	0 1	35 0	−	−	+	+	−	++	38 1
Potassium stearate	0 5	35 0					−	−	
" "	0 1	38 1	++	−	+++	++		+++	
" "	0 2	33 3	++	−	+++	++	+	+++	45 3
Glycerol mono oleate	0 5	28 5	−	−	−	++		++	
" "	0 1	29 2	+	−	−	+++	−	+++	
Sodium oleyl sulphate	0 5*	28 5	−	−	−	++		++	
Potassium oleate	0 2	29 2	−	−	+	+		+++	26 8
" "	0 1	28 2	−	−	−	−	−	−	
Sodium oleate	0 5	27 2	−	−	−	−			
" "	0 1	27 8	−	−	−	++		+++	
Sodium lauryl sulphate	0 5	27 8	−	−	−	++		++	32 0
Lecithin	0 5	26 5	−	−	−				28 2
"	0 1	25 5	−	−	−	++	+	++	
Triethanolamine lauryl sul phate	0 5	23 5	−	−	−	+++	+	+++	31 9
"	0 5	24 2	−	−	−				24 2
B.A I synthetic		73 0	+++	+++	+++	+++	+++	+++	
Glycerol broth		49 1†	+++	+++	+++				
Egg yolk (Besredka's medium)		34 7†	+++	+++	+++				

+ + + Very good growth
+ + Good growth
+ Slight growth
− No growth

* This is greater than the saturation solubility
† Surface not cleaned before measurement of surface tension

liquid media it seems that growth is adversely affected when the surface tension is reduced below about 40 dynes/cm and that there is no growth at all below about 30 dynes/cm On solidification by

means of 2 per cent. agar, growth is often permitted on media which previously inhibited. This point, which is of some interest, is discussed below.

Some differences were observed between the organisms' studied, growth of *Myco. tuberculosis*, human type, being much more readily inhibited than that of the other two, which behaved rather similarly. Growth occurred as a surface pellicle in all cases except with Besredka's egg-yolk medium, where it occurred throughout and was very abundant.

In a number of cases the surface tension was measured at intervals during growth and it was invariably found that the surface tension rose, ultimately to that of the medium before addition of the depressant, showing conclusively that the depressant was being adsorbed on the surface of the growing bacteria.

A modified B.A.I. synthetic medium containing no ferric citrate and only one-half the previous amount of glycerol gave results, both for growth and for surface tension, identical with those on one of the usual composition, sodium and potassium oleate, sodium and potassium stearate and sodium ricinoleate being used as surface-active substances.

Discussion

According to Larson and his co-workers (1919, 1921-22, 1922-23) *Myco. tuberculosis* will grow throughout the body of the medium when the surface tension is depressed from 59 dynes/cm., the value for ordinary broth, to 40-45 dynes/cm., using soaps of ricinoleic acid and stearic acid or bile. Cooper (1930) found that progressively increasing amounts of sodium ricinoleate, with accompanying decrease in surface tension, caused a corresponding inhibition of the rate of growth. In his experiments no sign of growth beneath the surface was observed, a finding confirmed by the present work.

Larson claimed that better wetting of the organisms by the medium creates better nutritive conditions, but Freedlander (1940) believes that wetting agents in general possess the property of penetrating and emulsifying surface films of fatty substances essential for growth, thus creating unfavourable conditions.

The results of our investigation show that surface-active substances are adsorbed or incorporated in some way by the bacteria, even when these are growing apparently normally. Such adsorption is not unexpected, since the surface of these organisms is known to be of a waxy or lipoidal nature, and from the Gibbs equation adsorption would increase with decreasing surface tension. Presumably there is a limit to the amount of disturbance necessarily caused by this adsorption which the bacterium can withstand before losing its power to reproduce. The present experiments indicate that with *Myco. tuberculosis*, human type, this limit is reached when the surface tension is about 42 dynes/cm., whereas with the avian type and

with *Myco. phlei* a surface tension of about 30 dynes/cm. is necessary. These differences may arise either from a difference in the boundary membranes leading to different adsorptions (or rates of penetration), or to varying susceptibility to "poisoning" in general.

Solidification of inhibiting liquid media by the addition of 2 per cent. agar permits growth to occur in some cases, as pointed out above. This might be explained in two ways. The surface-active compound might combine to some extent with the agar, reducing its effective concentration and surface activity to a degree which permits growth. The fact that the surface tension is in general raised by the addition of agar lends some support to this view, particularly as the differences would be enhanced if the liquid and solid media had been compared at the same temperature. The alternative explanation is that solidification, by eliminating convection, reduces diffusion of the surface-active compound to the surface of the bacterium and, if this reduction is sufficient, growth would again be permitted. It is of course possible that both factors are operative.

The exceptional behaviour of Besredka's egg-yolk medium in exhibiting flocculent growth throughout the medium may be due to the presence in this system of a mixture of proteins and phospho-lipoids, the low surface tension arising from the latter. Unfortunately no pure specimens of these phospho-lipoids were available to enable this hypothesis to be tested.

Summary

1. The influence of fourteen surface-active compounds giving surface tensions in the range 50-24 dynes/cm. in a synthetic fluid medium upon the growth of three strains of acid-fast bacteria has been investigated.

2. The growth of *Mycobacterium tuberculosis*, human type, was inhibited at surface tensions below about 42 dynes/cm., whereas with *Mycobacterium tuberculosis*, avian type, and *Mycobacterium phlei* growth was inhibited only at surface tensions below about 30 dynes/cm.

3. The chemical nature of the depressant used did not appear to be a relevant factor.

4. Media containing depressants showed an increase in surface tension during growth, tending to the value of the untreated medium, showing that these compounds were adsorbed by the bacteria.

5. Solidification of growth-inhibiting liquid media by 2 per cent. agar permitted growth to occur in some cases. Possible reasons for this are discussed.

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NEUTRALISATION OF *C. DIPHTHERIÆ* TYPE TOXINS WITH STANDARD ANTITOXIN, AS DETERMINED BY SKIN REACTIONS IN GUINEA-PIGS

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AN account was given in a previous communication (Zinnemann, 1943) of the toxin production of the three types of *C. diphtheriæ* in a medium containing iron greatly in excess of the optimum. The toxins formed were measured by their minimal reacting dose. The average amount of toxin constituting one M.R.D. for the guinea-pig was expressed in terms of multiples of the average M.R.D. of 16 experiments with a standard P.W. 8 strain. The mean M.R.D. for *gravis* strains was 1·7 times, for *intermedius* 3·4 times and for *mitis* 4·1 times greater than for the standard P.W. 8 strain.

The purpose of the present experiments is to establish the point of neutralisation of such toxins by standard antitoxin.

Following upon the discovery of the three main types of *C. diphtheriæ* a number of investigators (Parish *et al.*, 1932; Povitzky *et al.*, 1933; Priggc, 1935.36; Zinnemann and Zinnemann, 1939) established that P.W. 8 antitoxin afforded equal protection to guinea-pigs injected with toxins or cultures of *gravis* and *mitis* strains. Etris (1934) published figures which showed that one *gravis* antitoxin out of 4 gave better protection against *gravis* cultures than P.W. 8 antitoxin, if antitoxin doses were used which were either lower or only slightly higher than the minimal amount required for protection. The controversy was again taken up by Clauberg (1939), who contended that in the white rabbit's skin the amount of standard antitoxin required to neutralise "biologically equivalent doses" of type toxin was two to three times greater for *gravis* than for *mitis* toxin. He concluded that the *C. diphtheriæ* type strains either differ quantitatively in the relative amounts of toxin and toxoid which they produce, or produce a type-specific toxin component, which can be neutralised only by excess of antitoxin, acting as a kind of "carrier" for a hypothetical antibody against the supposed type-specific toxin component. Mueller (1941) described two experiments in which antitoxin neutralised toxins produced by a P.W. 8 and a *gravis* strain equally well. The iron content of the media was adjusted to exceed the optimum value. Neutralisation of toxin was determined by survival of guinea-pigs which had received 40 M.L.D. of toxin prior to the administration of 0·6-1·7 units of antitoxin.

It was thought that differences in the neutralisation of type toxins, if they existed, would be detected more readily if neutralisation were determined by a very sensitive method, using minute doses of

toxin and antitoxin. Römer's (1909) intracutaneous method in the white guinea-pig was adopted although, according to Jensen (1933), this test is less sensitive than that in the white rabbit. Technical difficulties determined the choice of the guinea-pig method. The results of two experiments, however, were checked on the white rabbit's skin.

Method

The technique previously described (Zinnemann, 1943) for the production of type toxins was followed. The earlier experiments had shown that a broth based on meat extract prepared at 48° C. never had an iron content below 2 μg/c.c. or above 7 μg/c.c. Both values being greatly in excess of the optimum and the toxin production not being appreciably affected within this range, it was not thought necessary to determine the iron content of the various batches of broth used in the present experiments. In the previous report the depth of medium in the bottles used for toxin production was not stated. In both series of experiments the layer of medium was approximately 1 cm. deep.

Each experiment consisted of a preliminary toxin titration and subsequent neutralisation experiment, using 4 strains consisting of *gravis, mitis* and *intermedius* and P.W. 8 " Elstree 110 ". The M.R.D. of the toxins used in each experiment was established on the shaved skin of the abdomen of white guinea-pigs. To exclude reactions peculiar to individual animals, only 4 injections were given to each guinea-pig, the same dilutions of each of the 4 toxins being tested on the same animal. This principle was also used in the neutralisation experiments, the same dilutions of antitoxin mixed with 1 M.R.D. of each of the toxins being tested on one guinea-pig. The technique commonly used for titration by flocculation was followed in these neutralisation experiments, *i.e.* setting up toxin-antitoxin mixtures with progressively decreasing amounts of antitoxin. The toxin-antitoxin mixtures were left to stand for 2 hours in the dark, at room temperature, before injection. Preliminary experiments showed that amounts of antitoxin from 0·00020 to 0·00001 I.U. were required to neutralise 1 M.R.D. of the 4 toxins tested in each experiment. The 10 antitoxin dilutions employed, made up to equal volumes with saline, contained the following amounts of antitoxin per 0·2 c.c. of toxin-antitoxin mixture injected : 0·00020, 0·00016, 0·00014, 0·00012, 0·00010, 0·00008, 0·00006, 0·00004, 0·00002 and 0·00001 I.U. Such a series was considered sufficient for the detection of increased fixation of antitoxin, should one of the type toxins in the experiment require a significantly larger dose of antitoxin for neutralisation than the remainder. Neutralisation was considered to have taken place if the local reaction produced by the injected toxin-antitoxin mixture had a diameter considerably less than 10 mm. or if the skin reaction was no longer red but yellowish in colour, or was absent altogether. The standard antitoxin used in all experiments was obtained from the National Institute for Medical Research (no. DF 12, containing 100 I.U. per c.c. : $\dfrac{\text{in-vivo}}{\text{in-vitro}}$ ratio = 1).

Experiments on white guinea-pigs

Twelve neutralisation experiments were carried out. The results are recorded in table I. In 7 experiments the *gravis, mitis, intermedius* and P.W. 8 toxins in each experiment were neutralised by the same amount of antitoxin, although the amount of antitoxin differs from one experiment to another. In these 7 experiments neutralisation of

TABLE I

*The neutralisation of 1 M.R.D. of type strain toxins and P.W. 8 strain
"Elstree 110" toxin with standard antitoxin on white guinea-pigs*

Expt. no.	Strain from which toxin was prepared		Source	Amount of antitoxin (I.U./0.2 c.c.) necessary to neutralise 1 M.R.D.
1	SS 227	G	Leeds	0.00004
	211	M	,,	0.00004
	263	I	,,	0.00004
	P.W. 6		...	0.00004
2	HC 447	G	Hull	0.00010
	SS 290	M	Leeds	0.00010
	0567/4	I	,,	0.00010
	P.W. 6		...	0.00010
3	244	G	Leeds	0.00014
	7264/1	M	,,	0.00012
	162	I	,,	0.00012
	P.W. 6		...	0.00014
4	G 2	G	Glasgow	0.00014
	11/5	M	Bristol	0.00014
	214	I	Leeds	0.00010
	P.W. 6		...	0.00014
5	577/5	G	Leeds	0.00010
	485	M	,,	0.00010
	236	I	,,	0.00010
	P.W. 6		...	0.00010
6	6440	G	Leeds	0.00010
	6128/3	M	,,	0.00010
	6049/2	I	,,	0.00010
	P.W. 8		...	0.00010
7	243	G	Leeds	0.00010
	6020	M	,,	0.00006
	476	I	,,	0.00010
	P.W. 8		...	0.00010
8	6578	G	Leeds	0.00006
	6128/3	M	,,	0.00006
	6049/2	I	,,	0.00006
	P.W. 8		...	0.00006
9	261 A	G	Halifax, Nova Scotia	0.00010
	253 A	M	,, ,, ,,	0.00010
	112 A	I	,, ,, ,,	0.00010
	P.W. 8		...	0.00010
10	HC 453 *		Hull	0.00006
	7446	M	Leeds	0.00008
	6630/2	I	,,	0.00006
	P.W. 6		...	0.00006
11	6653/3	G	Leeds	0.00004
	6468	M	,,	0.00004
	6438	I	,,	0.00004
	P.W. 6		...	0.00004
12	Rice	G	Dundee	0.00008
	6612	M	Leeds	0.00006
	470	I	,,	0.00010
	P.W. 8		...	0.00010

* Atypical.

all 4 toxins took place on the same guinea-pig. This is obviously a much higher proportion than could have been expected considering the variation of the neutralising dose for P.W. 8 toxin in the whole series. In 5 experiments (nos. 3, 4, 7, 10 and 12) different amounts of antitoxin were required. The differences were, however, mostly slight and were well within the limits of experimental error. It should be borne in mind that a direct comparison of the values in the 5 experiments showing discrepancies is not justified, since the values were obtained on different guinea-pigs within these experiments. There is no regularity or specificity in these slight discrepancies. In expt. 3 the *gravis* and P.W. 8 toxins required more antitoxin for neutralisation than did the *mitis* and *intermedius* toxins, whilst in expt. 4 the same was true for the *gravis*, *mitis* and P.W. 8 toxins as compared with the *intermedius* toxin. In expt. 7 the *mitis* toxin requires less and in expt. 10 more antitoxin than the others. In expt. 12 the *intermedius* and P.W. 8 toxins require more antitoxin than the *gravis* and *mitis* toxins for neutralisation. None of these differences, however, are significant.

Expts. 10 and 12 are of special interest. Strain HC 453 is an atypical strain—type IV of Wright and Christison's (1935) classification. Such strains correspond to the *gravis* type in all respects except for their failure to ferment starch. Strain " Rice " in expt. 12 is a *gravis* strain (Dundee variant). The characteristics of these strains were described by Robertson (1943) and were also mentioned by Johnstone and Zinnemann (1943). Toxin production by such atypical strains and *gravis* strains (Dundee variant) was shown to be not higher than that produced by *mitis* strains (Zinnemann, 1943). Neutralisation of toxins from such strains takes place in the same way as for toxins from *gravis*, *mitis* and *intermedius* strains and the P.W. 8 strain " Elstree 110 ".

Experiments on white rabbits

When it became obvious that there was divergence from Clauberg's results, two of the experiments set out in table I (nos. 7 and 8) were checked on shaved white rabbits, since this author used Jensen's method. The M.R.D. of each toxin was re-determined for the rabbit's skin. This preliminary toxin titration and the neutralisation of the M.R.D. of the 4 toxins thus established were carried out on the same animal in each experiment. Table II gives the values for the neutralisation of 1 M.R.D. of the two groups of toxins tested. The results confirm those of the experiments on guinea-pigs.

Thus it can be stated that all the diphtheria toxins tested, *i.e.* those produced by a number of *gravis*, *mitis* and *intermedius* strains, by 1 variant of *gravis* and 1 atypical strain as well as by strain P.W. 8 " Elstree 110 ", could be neutralised equally well by standard P.W. 8 antitoxin.

TABLE II

Two toxin neutralisation experiments on the white rabbit

Expt. no.	Strains from which toxin was prepared		Source	Amount of antitoxin (I.U./0·2 c.c.) necessary to neutralise 1 M R D.
7	243 ·	G	Leeds	0·00004
	6020	M	,,	0·00004
	478	I	,,	0·00006
	P.W. 8		...	0·00004
8	6578	G	Leeds	0·00010
	6123/3	M	,,	0·00010
	6049/2	I	,,	0·00010
	P.W. 8		...	0·00010

Extended observations on toxin production by the different types of C. diphtheriæ

The experiments just described extended the observations on toxin production reported in the previous communication, in which a small group of atypical and unusual strains had been excluded from the general conclusions. For this reason expts. 10 and 12 are excluded from table III, which analyses the M.R.D. values of the toxins as in the previous series. A number of strains in the present series had been used in the experiments reported earlier, but except for expt. 1 these strains were grouped in different combinations, or with other strains not previously used. In expts. 6 and 8 the *mitis* and *intermedius* strains were identical. The weighted means for the 10 *gravis*, 10 *mitis* and 10 *intermedius* toxins are all somewhat higher than in the first series. The values were then 0·00165 for *gravis*, 0·00413 for *mitis* and 0·0034 for *intermedius* toxin, all figures for the M.R.D. of the P.W. 8 toxins being adjusted to 0·001. If the two sets of experiments are considered together, the resulting weighted means for the 26 experiments are almost identical with those obtained for the 16 experiments in the first communication.

Discussion

The results of this investigation confirm earlier observations (Zinnemann and Zinnemann) and those of other workers in this field (Parish *et al.*; Povitzky *et al.*; Priggo; Mueller), but Clauberg's results and conclusions are in complete opposition, although he employed similar methods. An explanation for this discrepancy can perhaps be found in the tables contained in his paper. From these it seems obvious that his " biologically equivalent doses " of toxin, which he neutralised, were arbitrarily chosen for the similarity of the skin reactions produced by *mitis* and *gravis* toxins. They do not seem to correspond to minimal reacting doses (M.R.D.) or any other standard dose, but they constitute undetermined multiples of 1 M.R.D. A rather large experimental error is thus introduced, since

it is possible to produce identical " biological reactions " with different multiples of 1 M.R.D. These, in their turn, will require different amounts of antitoxin for their neutralisation.

For the same reason his figures relating to the amount of toxin

TABLE III

Toxin production of type strains and P.W. 8 strain " Elstree 110 "

Expt. no.	Strain from which toxin was prepared		Source	M.R.D. relative to P.W. 8 = 0·001 c.c.			
				Gravis	*Mitis*	*Intermedius*	P.W. 8
1	SS 227	G	Leeds	0·002	...	...	...
	211	M	,,	...	0·01	...	...
	263	I	,,	...	...	0·01	...
	P.W. 8		...	...	...	...	0·001
2	HC 447	G	Hull	0·004	...	...	...
	SS 290	M	Leeds	...	0·01	...	...
	6587/4	I	,,	...	...	0·01	...
	P.W. 8		...	...	...	...	0·001
3	244	G	Leeds	0·0005	...	...	...
	7264/1	M	,,	...	0·002	...	...
	182	I	,,	...	...	0·002	...
	P.W. 8		...	...	...	...	0·001
4	G 2	G	Glasgow	0·002	...	...	...
	11/5	M	Bristol	...	0·002	...	...
	214	I	Leeds	...	...	0·002	...
	P.W. 8		...	...	...	...	0·001
5	577/5	G	Leeds	0·0005	...	...	...
	485	M	,,	...	0·003	...	...
	238	I	,,	...	...	0·0017	...
	P.W. 8		...	...	...	...	0·001
6	6440	G	Leeds	0·0017	...	...	...
	6128/3	M	,,	...	0·002	...	...
	6049/2	I	,,	...	...	0·0014	...
	P.W. 8		...	...	...	...	0·001
7	243	G	Leeds	0·0027	...	...	...
	6020	M	,,	...	0·0067	...	...
	478	I	,,	...	...	0·0019	...
	P.W. 8		...	...	...	...	0·001
8	6578	G	Leeds	0·004	...	...	...
	6128/3	M	,,	...	0·008	...	...
	6049/2	I	,,	...	...	*0·002*	...
	P.W. 8		...	...	...	...	0·001
9	261 A	G	Halifax, Nova Scotia	0·004	...	...	...
	253 A	M	,,	...	0·008	...	...
	112 A	I	,,	...	...	*0·008*	...
	P.W. 8		...	...	...	...	0·001
10	6653/3	G	Leeds	0·0005	...	...	...
	6488	M	,,	...	0·002	...	...
	6438	I	,,	...	...	0·001	...
	P.W. 8		...	...	...	...	0·001
	Total			0·0219	0·0537	0·040	0·010
	Mean			0·00219	0·00537	0·004	0·001

produced by type strains of *C. diphtheriæ* should be treated with caution, although they fall into line with the results of Mueller and the writer. It is unlikely that the strains used in these experiments and those of Clauberg had different properties. This view is strengthened by the fact that, besides those isolated in the Leeds area, strains were included in the present investigation which were obtained from other areas of the British Isles, as well as three strains from Halifax, Nova Scotia. Outbreaks of a severity comparable to that of European epidemics had occurred in Halifax (Wheeler and Morton, 1942). The toxins of these three strains were found to be in no way different from those of the Leeds strains, either in amount produced or in the extent of their neutralisation with antitoxin.

Clauberg (1939) and O'Meara (1940) put forward hypotheses assuming the existence of type-specific toxin components. Clauberg's communication has already been discussed, and O'Meara's basic observations could not be confirmed by Frobisher and Mauss (1943), Povitzky (quoted by Frobisher, 1943) or Zinnemann (1943). Until more convincing evidence is available, our knowledge of the facts underlying the differences in pathogenicity of the *C. diphtheriæ* types remains restricted mainly to the larger amount of toxin produced by *gravis* and *intermedius* strains, particularly under conditions similar to those present in the human and animal body. This idea, first suggested by Povitzky *et al.*, was demonstrated experimentally by Irene Zinnemann and Glusman (*vide* Zinnemann, 1943, p. 276), Clauberg, Mueller, Zinnemann (1943), and recently in a very neat way by Ørskov *et al.* (1944). Mueller considerably increased our knowledge by emphasising the importance of excess of iron in demonstrating the quantitative differences in toxin production. Ørskov *et al.* state that besides the larger amount of toxin produced in the tissues, there is also a marked inhibition of phagocytosis by *gravis* strains. This is confirmed in a forthcoming paper by Orr-Ewing (1946). Absence of phagocytosis might be expected to facilitate invasion of the animal body by *gravis* strains, but these workers did not succeed in isolating *C. diphtheriæ* of any type from the internal organs of guinea-pigs infected subcutaneously. Several observers, on the other hand, have demonstrated the presence of diphtheria bacilli in these circumstances (Robinson and Marshall, 1934; Gundel and Erzin, 1935; Gins, 1935-36; Kroomor, 1936-37; Clauberg and Plenge, 1937; Zinnemann and Zinnemann, 1939; Zinnemann, 1940). It is difficult to assess at present whether, and if so to what extent, an invasion of the tissues and internal organs plays a part in malignant diphtheria. Considerably more animal experiments and post-mortem investigations on severe clinical cases are necessary to elucidate this point.

Summary

1. Toxins of *C. diphtheriæ gravis, mitis* and *intermedius* strains as well as of P.W. 8 strain " Elstree 110 ", produced in a medium containing

a large excess of iron, are neutralised equally well by standard P.W. 8 antitoxin. The determination of the M.R.D. of the toxins and their neutralisation was carried out on the white guinea-pig's skin.

2. Toxins of a "Dundee variant" of the *gravis* type and of an atypical strain were neutralised in the same way.

3. Two experiments repeated on the white rabbit's skin confirmed the results obtained on the guinea-pig.

4. Our present knowledge of the facts underlying the association of the *C. diphtheriæ* types with clinical cases of differing severity is discussed.

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A HISTOLOGICAL AND BACTERIOLOGICAL STUDY OF HEALING BURNS WITH AN ENQUIRY INTO THE SIGNIFICANCE OF LOCAL INFECTION

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(Plates III-VII)

Three factors determine the mode and rate of healing of a burn : (1) the severity and therefore the initial depth of injury, (2) the degree of mechanical or chemical trauma to the surface during treatment and (3) the presence or absence of infection. In clinical practice it is often impossible to assess the part played by each of these factors when a burn fails to heal in the expected time. If, however, the initial depth of injury be known (*vide infra*) and further surface damage avoided by the use of bland applications (Cannon and Cope, 1943 ; McLure and Lam, 1943), the effects of the presence of pathogenic bacteria can be studied more precisely, including the way in which the inflammatory reaction varies in second and third degree burns.

Definitions of the term " wound infection " vary ; we have used it in the main to mean the recovery of pathogenic organisms from an inflamed surface. Many workers, notably Aldrich (1933), Cruickshank (1935) and Clark *et al.* (1943), have shown the frequency with which hæmolytic streptococci invade burns and have stressed the importance of this organism. *Staphylococcus aureus* is also a common invader (Heggie and Heggie, 1942 ; Bodenham, 1943). Bodenham states that hæmolytic streptococci are responsible for the most serious of the disturbances, but *Staph. aureus* also can produce severe local reactions.

Our own method of investigation consisted in making clinical observations at the time of the initial cleansing (which was as gentle as possible, using soap and saline), while also taking swabs and biopsies. Similar observations were made at daily or longer intervals. In nearly all cases the dressings consisted of jelonet covered by an occlusive pressure dressing. Sulphonamides were used if hæmolytic streptococci were recovered.

Initial clinical appearances

Gradations of severity ranged from intact blisters through a red, raw, moist surface which blanched on compression to more severe grades where the surface either appeared purpuric or was pale and dry. The nature of the exudate which formed after cleansing and drying was noted. In the least severe of the open wounds it was serous ; in more severe cases it was often bloodstained and

the exuded fibrinogen tended to coagulate. Where the surface was pale, little or no exudate formed. A large intact blister always implied a second degree burn, a red raw surface usually but not always. Purpura appearing soon after injury or within 24 hours occurred in both second and third degree burns. Pale, dry, non-exuding surfaces almost always meant third degree burns, except in the case of a few flame burns. We confirmed the observation that flame burns produce less surface exudate than scalds, that there is less likelihood of large tense blisters being formed and that in general they give an appearance of deeper injury than is in fact the case. Thus on one occasion the thickened skin over the tibial tuberosity showed manifest superficial charring, yet second degree type healing occurred in the usual time.

Bacteriological technique

Swabs were usually taken immediately after the initial cleansing and again whenever the wound was exposed for examination. They were taken so as to collect the flora from a wide area and were plated without delay on heated and unheated blood agar and incubated for 24 hours at 37° C. The coagulase reaction was done on all staphylococci and the soluble haemolysin test on all streptococci suspected of being haemolytic. In many cases different parts of the wound were examined separately.

Biopsy technique

Biopsies were taken by means of a sharp cork borer ¼ in. in diameter, which was found to give a sufficient amount of tissue. If the surrounding parts healed as a second degree lesion, the biopsy scar was of pin-head size. If taken from a third degree area no additional scar was evident. The tissue was fixed in 10 per cent. formol-saline, sections were cut serially and stained with haematoxylin and eosin, by various other methods for collagen, elastic fibres and fibrin, and for organisms by Gram's method. Biopsies were taken from many other burns besides those reported here. They were also taken from a number of cases during the subsequent course. So far as we are aware, no systematic histological study of this kind has as yet been reported. There have, however, been several papers dealing with regeneration of donor sites after skin grafting (Cannon and Cope, 1943; Hirshfeld et al., 1943; and others), a process which to some extent simulates the healing of second degree burns.

Scrape biopsies. In several cases where it was deemed inadvisable to take a biopsy, the surface of the burn was firmly scraped with a scalpel and smears made of the material. The object was to find out whether there was growing epithelium—i.e. evidence of healing—in areas where this was doubtful or where inflammatory exudate obscured the surface. In these smears, if epithelial cells were present, they were easily made out as masses of squames and this was taken as evidence of attempted healing.

HISTOLOGICAL OBSERVATIONS

First degree burns

These include all burns from simple erythema to a " peau d'orange " appearance and involve partial destruction of the surface epithelium, although the stratum corneum remains intact.

Histology. One biopsy was taken from such a burn. It shows partial heat necrosis of the epidermis (fig. 1), the cells lying between the dermic papillae appearing viable and very early intra-epidermal

HISTOLOGY OF HEALING BURNS

Fig. 1.—Severe 1st degree burn five hours after injury, showing partial destruction of epidermis with intact though thickened stratum corneum. No blisters formed. Healing occurred in 2 or 3 days, with scaling and pigmentation. H. and E. ×90.

Fig. 2.—Typical blister skin from 2nd degree scald a few hours after injury. The whole epidermis is necrotic and has been lifted off the dermis in one sheet by blister fluid. Note intact stratum corneum. H. and E. ×90.
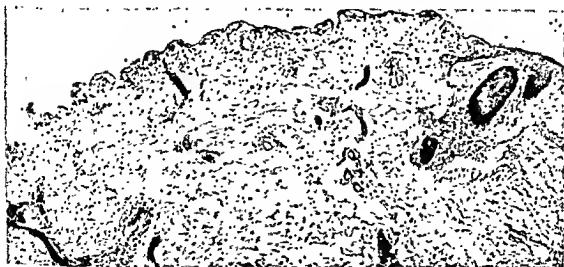
Fig. 3.—Typical 2nd degree burn six hours after injury, showing total loss of epidermis. Dermal papillæ easily made out, with surviving epithelium in sweat ducts and hair follicles. Healed in 9-10 days. Hæmolytic streptococci were recovered from this area on the 3rd, 4th and 5th days. H. and E. ×50.

vesicle formation is seen. The stratum corneum is intact, but beginning to show some separation into its component layers. There is marked capillary congestion of the dermal papillæ but no cellular infiltration.

Second degree burns

Here the surface epithelium is totally destroyed, laying bare the dermis, with varying degrees of incomplete necrosis of hair follicles and sweat ducts. Such areas may be covered by blisters.

Histology. Epithelium. The surface epidermis, apart from a few necrotic cells lying between the dermal papillæ in an occasional biopsy, is wholly lacking (figs. 3-5). The blister skin (fig. 2) always consists of the whole epidermis and never of the stratum corneum alone, as apparently may occur in burns of the hands and feet (Patey and Scarff, 1944-45).

The hair follicles show a gradation from above downwards in the damage to the cells composing them. In a typical section the upper third or so of cells (fig. 4) shows complete necrosis with loss of all detail, resulting in a homogeneous· strongly eosinophilic reaction. Further from the surface various types of cell damage are encountered. These have been described in detail by Leach *et al.* (1943-44). Nuclear pyknosis is common, as are also nuclear shrinkage and vacuolation. Karyorrhexis is less frequent. A common finding is linear shrivelling of the cytoplasm and nucleus, usually at right angles to the surface (figs. 6 and 7). Cells showing these changes gradually give place to those which are histologically normal. In many biopsies it is impossible to say with certainty whether or not the epithelium has undergone an irreversible change. It is very probable also that in some cases the epithelium may undergo further retrogressive changes later.

The cells lining the sweat ducts show changes similar to those described above. The transition from obviously necrotic to apparently normal cells is often striking.

Corium. As a rule the outlines of the dermal papillæ are easily seen (figs. 3 and 5). In milder burns, where the epidermis has been lifted off entire as a blister, little obvious damage to the collagen is seen apart from a very thin crust of coagulated collagen. In more severe burns the whole of the collagen in the papillæ has lost its fibrillary structure and become a homogeneous mass, frequently staining basophilic with hæmatoxylin or yellowish with van Gieson (fig. 3). The fibrocyte nuclei show, when damaged, very bizarre straggling shapes even in mild burns and disappear completely by lysis in the more severe. In the milder burns there is marked congestion of the superficial capillaries, in the more severe, intravascular hæmolysis is present and the capillaries are seen as rounded spaces filled with eosinophilic masses, possibly thrombi. The nuclei of the endothelial cells have vanished in such cases. In areas of milder damage partial loosening of the endothelial cells can be seen, with

varying damage to their nuclei. There is no increase in the number of polymorphs in the vessels, even in burns up to 6 hours old. Escape of red cells into the surrounding connective tissue is usually slight. In milder second degree burns there is no evidence of damage to the elastic fibres, but in some of the more severe there is loss of sharpness in the staining of the vertical fibrils in the papillæ. Fibrin is never found in the interstices of the collagen, but often forms a thin pellicle on the surface of the papillæ (fig. 5). A few scattered polymorphs are encountered in some cases. They are never seen on the surface in initial biopsies, but it must be remembered that these were taken only after cleaning the wound with soapy saline.

Third degree burns

Biopsies were taken from early third degree burns only where there was clinical doubt as to the degree of injury. Their characteristic feature is the absence of all histologically normal epithelial elements save in some cases a deep sweat coil lying in the subcutaneous fat. The damage to the corium varies. Dermal papillæ are still sometimes visible, though usually flattened, and the collagen is changed into a more or less homogeneous hyaline layer. In the deeper dermis the bundles of collagen are seen as broad hyalinised bands, widely separated, presumably by œdema. A change in the normal acidophilic reaction of the collagen is often marked. The tissues are notably avascular and there is no evidence of infiltration by polymorphs or lymphocytes (figs. 6 and 7).

Healing of second degree burns

This was studied by means of a few biopsies taken at intervals of from 3 to 12 days after injury (figs. 8-14). The findings confirm the fact that healing takes place from viable cells in hair follicles and sweat ducts ; there is no evidence in our material of participation by sebaceous glands or sweat coils. The mechanism consists in the formation of a solid vertical column of cells which pushes its way to the surface, where the cells spread out over and between the dermal papillæ. The spreading cells, at first quite flat and in a single layer (fig. 13), are closely applied to the surface collagen and appear to work their way under any inflammatory exudate that is present. In some sections, however, the apposition of epithelium and dermis is unsuccessful and results in small masses of epithelial cells lying detached in or on a purulent exudate. In the hair follicles it is of course the peripheral cells which multiply, occasional mitotic figures being seen among them. A central solid core of epithelium results which gradually grows (or is pushed) to the surface. In the sweat ducts the first change is a metaplasia of the lining epithelium into angular squamous types which fill the lumen, forming a solid column

Histology of Healing Burns

Fig 4.—Second degree burn five hours after injury. High power view of hair follicle. Note necrosis in upper third with varying degrees of degenerative change in cells below. Viable cells with normal nuclei can be made out in centre of lower half. H. and E. ×250.

Fig 6.—Third degree burn six hours after injury. The dermal papillæ, denuded of epithelium, are flattened and the cells in sweat ducts and coils and in hair follicles show early necrosis. Note linear shrivelling of nuclei in sweat duct. The dermis is avascular and shows no sign of leucocytic infiltration. Burn failed to heal and had to be grafted. H. and E. ×90.
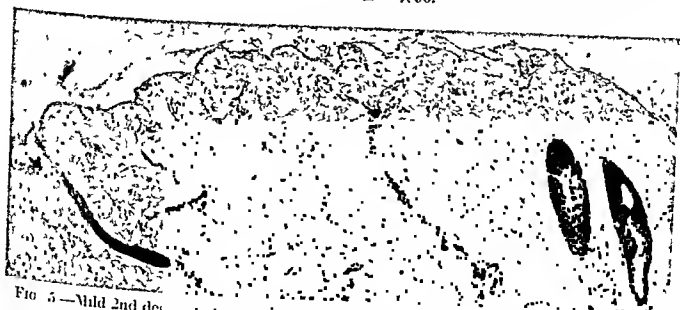
Fig 5.—Mild 2nd deg. ... four hours after injury. Dermal papillæ congested but otherwise little damaged. Hair follicles and sweat ducts are seen with surviving epithelial cells. Considerable fibrinous exudate on surface. Staph. aureus recovered on first dressing (4th day) and Staph. aureus and hæmolytic streptococci on second dressing (8th day). Healing occurred in 8-9 days. H. and E. ×90.

of cells arranged in a mosaic (figs. 9, 10 and 12). In both proliferating hair follicles and sweat ducts increase of cell size and nuclear hyperchromatism are striking features. As the hollows between the dermal papillæ become filled with epithelial cells there is a gradual change from a single layer of flat scales to more fleshy types two or more cells deep, until at length a stratified epithelium is again formed. The cells in the first stages are arranged rather loosely, but gradually become more closely knit. The fibrinous exudate covering an inflamed surface appears to act as a protective covering until epithelial cells have spread between it and the underlying papillæ. Bacteria are always noted on the surface of this exudate and only occasionally between the fibrinous strands. Where infection is heavy the zones from above downwards are organisms, fibrin, leucocytes, but naturally these merge with each other in varying proportions (fig. 15).

Healing of third degree burns

These heal slowly from the edges over pre-formed granulation tissue. Very occasionally an isolated patch of epithelium appears on the granulating surface, presumably derived from some deep-seated source of viable epithelial cells. In general, these small areas, which are seen only in the milder grades, contribute little to the healing process, in contrast to the multifocal and rapid healing of second degree burns.

CASE REPORTS

Case I. Child with extensive blistered sealds. One blister on the wrist, 1 in. in diameter, had ruptured, exposing a pink velvety surface. This area was chosen for study. Swabs taken after cleansing gave a growth of hæmolytic streptococci. On the 2nd day an originally vesiculated area surrounding the wound was also raw and clinically infected, with a marked " flare " 6 mm. wide, 2 mm. of it affecting the unburnt skin. The original wound was covered with thick fibrinopurulent exudate. Hæmolytic streptococci were still present, but biopsy showed proliferation of sweat ducts and hair follicles, with epithelial cells beginning to cover the denuded surface. Powdered sulphonamide was applied at this and subsequent dressings. By the 4th day the " flare " had largely faded and the " bloom " of new epithelium was just visible in areas not covered by exudate. Hæmolytic streptococci were still present and *Staph. aureus* now appeared. On the 6th day the wound was dry and scaling, although streptococci and scanty *Staph. aureus* were still present. By the 8th day the wound was healed.

This was a 2nd degree burn healing rapidly despite the presence of streptococci from a very early stage and later of *Staph. aureus*. Sulphanilamide applied on the 2nd day did not cause the disappearance of streptococci but may have reduced their number. Epithelial regeneration had started prior to the use of the drug.

Case II. Child with superficial burns of whole of left anterior thigh. After cleansing, swabs gave a growth of *Staph. albus*. The surface was raw and red, with some purpura at the periphery. Clinically a 2nd or early 3rd degree burn was suspected ; biopsies indicated a severe 2nd degree burn. Jelonet and pressure dressings were applied. On the 2nd day swabs from the lower thigh

gave a growth of *Staph. albus*, from the upper thigh a few hæmolytic streptococci also. Biopsies showed sweat duct and hair follicle proliferation almost reaching the surface. On the 5th day only *Staph. albus* and Gram-negative diplococci grew. Epithelium could be seen beginning to cover the thigh, even the originally purpuric areas, while an area of the lower thigh, though healing, was partly covered by a thin purulent layer.

This case represents the rather slower healing of a 2nd degree burn uncomplicated by gross infection, the slowness being due probably to the more severe and therefore deeper injury to the epithelial elements. The hæmolytic streptococci found on the 2nd day had disappeared by the 5th day without specific treatment : there was no clinical evidence of their presence at any time.

Case III. Child with large scald on upper half of right thigh. The lower 2 in. were clinically of 1st degree, the remainder mainly of 2nd degree, but a small central area about 1 in. in diameter was pale and clinically of 3rd degree. The peripheral parts of the 2nd degree burn, after cleaning and mopping dry, showed a watery " dew-drop " type of exudation. The more central areas exuded more slowly, the exudate promptly coagulating. The pale 3rd degree zone remained dry. A biopsy from the middle of the peripheral portion showed a typical 2nd degree burn, one from the middle of the pale area was of 3rd degree. A swab gave no growth. Treatment : jelonet and pressure dressing.

Four days after injury four gradations of healing could be distinguished : (1) 1st degree—healed with slight pigmentation ; (2) peripheral part of wound (of 2nd degree) covered by a thin layer of epithelium ; (3) more central but originally 2nd degree—punctate epithelialisation, *i.e.* regeneration less well advanced than in (2) (4) 3rd degree—covered by a thin greyish-white slough. Swabs from central and peripheral zones gave profuse growth of *Staph. aureus*, and a biopsy showed all epithelial elements completely necrotic.

By the 8th day all 2nd degree zones were equally well healed, leaving only the central area unhealed. Swabs from the central and peripheral areas yielded abundant *Staph. aureus* and hæmolytic streptococci. Sulphanilamide powder was applied on this day and subsequently. On the 10th day the original 3rd degree area was outlined by a flare with some re-ulceration of the adjacent healed 2nd degree burn. A few buds of granulation tissue were showing through the slough. *Staph. aureus* and streptococci still grew on culture. By the 18th day there was evidence of healing at the edges of the 3rd degree area. Culture gave *B. proteus* and *Staph. aureus*. On the 24th day the patient was discharged with a small unhealed area. *Staph. aureus* and hæmolytic streptococcus were still present.

This case is another example of slight and fairly severe 2nd degree burns healing in the presence of staphylococci and streptococci. It also shows that an infected 3rd degree area may become larger by ulceration of the surrounding 2nd degree zone.

Case IV. Adult with two separate burns of left shoulder and upper arm. The area over the shoulder consisted of a clinically 3rd degree central portion surrounded by a larger zone of probable 2nd degree. The burn on the arm was clinically 2nd degree. Biopsies were taken from : (1) the central pale area of the shoulder burn—3rd degree histologically ; (2) the peripheral zone of the shoulder burn—2nd degree histologically ; (3) the centre of the arm burn— 2nd degree histologically. Swabs from both wounds showed *Staph. albus* only. On the 5th day the dressings were removed. Both 2nd degree areas showed a comparable degree of healing, epithelium covering the whole surface (confirmed by scrape biopsy). The original pale white area on the shoulder was covered by a thick slough. By the 12th day the whole wound was healed, apart from the original 3rd degree area, which was still covered by moist slough.

Case V. Child with scalds of left thigh and leg and anterior aspect of right thigh. This last area was of severe 1st degree, showing a " peau d'orange "

HISTOLOGY OF HEALING BURNS

Fig 7.—Third degree burn four hours after injury Cells in hair follicles show irreversible damage Dermal papillæ can still be made out but show less of the normal fibrillary appearance—coagulated collagen Burn healed slowly from periphery over granulation tissue H and E ×90

Fig 9.—Healing 2nd degree burn (2nd day) 'Squamous metaplasia' of three sweat ducts deep in the corium Uninfected case healed in 7 8 days H and E ×180

Fig 8.—Healing 2nd degree burn (2nd day) Note commencement of healing from two sweat ducts but rest of surface still devoid of epidermis Healed in 6 7 days H and E ×90

Fig 10.—High power view of sweat duct to left of fig 9 Note mitotic figure on right border H and E ×400

Fig 11 —Healing 2nd degree burn (3rd day) Section taken at edge of burn with normal epidermis to right Cells from the mouths of two sweat ducts are beginning to spread over the surface, which is covered by a slight inflammatory exudate from which hæmolytic streptococci were recovered on this and on the 4th and 5th days Healed in 9 days H and E ×50

Fig 12 —High power view of sweat duct seen in fig 11 Note inflammatory infiltration H and E ×225

Fig 13 —Healing 2nd degree burn (2nd day) Part of hair follicle seen projecting above the surface and sending out epithelial cells over the dermis and beneath an inflammatory exudate Pure growth of hæmolytic streptococci recovered on 1st day and persisted throughout Healed in 8 days H and E ×120

appearance (fig. 1). The rest of the injury was moist and velvety red. A biopsy from the middle of the left thigh showed a typical 2nd degree injury. Swabs after cleansing gave a few colonies of *Staph. albus*. Jelonet and pressure dressings wore applied. On the 2nd day punctate regeneration of epithelium was visible. This was confirmed by biopsy on the 3rd day, when there was an inflammatory response over a large area of the leg and a swab showed a pure growth of hæmolytic streptococci. A biopsy of the inflamed area showed a thin fibrinous exudato over the regenerating epithelium, with moderate polymorpb infiltration and a few Gram-positive organisms on the surface of the fibrin. On the 4th day sulphonamides were applied and continued. On the 6th day the streptococci had disappeared, as had the inflammatory response, but *Staph. aureus* now appeared. Clinically, healing was everywhere equally advanced; the streptococci bad had no apparent retarding action. On the 7th day an acute inflammatory reaction was found in the upper part of the thigh, with a thick fibrinopurulent exudate over the regenerating epithelium and a surrounding "flare". A biopsy showed exudate lying on recently regenerated epithelium. There were masses of Gram-positivo cocci on the surface. Below this came a layer of fibrin, with small numbers of polymorphs; this gradually gave place to large numbers of polymorphs, and scanty fibrin. The epithelium, which was in an advanced stage of healing, was infiltrated by polymorphs and the dermic papillæ showed marked congestion, œdema and polymorph infiltration. A swab from this area yielded *Staph. aureus*, *Ps. pyocyanea* and diphtheroids. By the 9th day retrogression of tho inflammatory response was evident. By the 11th day all areas had healed, though *Staph. aureus* was still recovered.

This case illustrates the inflammatory response which occurs over hoaling epithelium, due to streptococci and, at a later stage, to *Staph. aureus*. These infections did not have any marked delaying action on epithelial regeneration.

Case VI. Child with scalds (due to tea) affecting the left arm and left side of the face and neck.

Arm. The surface was raw, red and weeping except for certain portions having a thin dry tan due to the tea. Swabs after cleansing gave no growth. A biopsy from the central most severely burnt area showed a 3rd degree burn. Ths burnt surface on the 1st day after injury showed marked purpuria mottling and swabs gave *Staph. aureus* on culture. On the 2nd day there was an appearance of early punctate epithelial regeneration at the periphery but no biopsy was dono to confirm this. By the 3rd day inflammatory changes were striking, with a surrounding "flare" and pus under some of the tanned areas. Swabs gave a profuse growth of *Staph. aureus*. On the 4th day the inflammation was still marked, with ulceration at the margin: this process continued until the 8th day. *Staph. aureus* remained throughout and in addition hæmolytic streptococci were recovered on the 4th day, and on the 5th and 6th days *B. coli* and *Staph. albus* as well. Healing took place slowly from the edge only and was not complete for several weeks; that is to say no 2nd degree typo healing occurred, such 2nd degree injury as was present boing dostroyed by ulceration spreading from tho central 3rd degree wound.

Face and neck. Two areas were involved, tho larger on the face, the smaller on the neck, both typical 2nd degree scalds, partly tanned by the tea. A swab after cleansing gave no growth. No biopsy was taken. On tbe 1st day a few colonies of *Staph. aureus* were recovered and subsequently the growth became more profuse, persisting until the 8th day. On the 3rd day there was a characteristic "flaro" and fibrinopurulent covering of the burn. By the 5th day, inflammation diminishing, there was evidence of epithelial regeneration at tho periphery: on this day and the next hæmolytic streptococci were recovered in addition to *Staph. aureus*. By the 7th day the inflammation had died down, revealing healed epithelium beneath.

This case illustrates once moro that 2nd degree injuries, here on the faco

and neck, may heal rapidly despite the presence of *Staph. aureus* and streptococci. The lesion on the arm, on the other hand, with a central 3rd degree area and a probable 2nd degree peripheral area, is an example of infection in a 3rd degree area spreading to 2nd degree areas and turning a 2nd degree into a 3rd degree injury.

Case VII.   Boy, with extensive scalds of left chest, axilla and anterior aspect of left arm, forearm and wrist. The axillary and peripheral part of the chest wounds and the wrist wound were clinically of 2nd degree, the remainder of 3rd degree. A biopsy from the centre of the arm confirmed the 3rd degree nature of this lesion. Punctate epithelial regeneration was visible in the 2nd degree areas on the 2nd day, *Staph. aureus*, hæmolytic streptócocci and coliform bacilli being recovered on the 3rd day from both the 2nd and 3rd degree areas. On the 4th day there was ulceration of a tongue of unburnt skin in the fold of the elbow extending from the arm burn. Sulphanilamide powder was applied. By the 8th day further spread of ulceration had stopped and healing was beginning. The 2nd degree burns were healed in some parts by the 10th and in others by the 12th day, *Staph. aureus* still being present in areas of the healing burn. The 3rd degree areas healed with considerable delay. *Staph. aureus* remained the dominant organism, hæmolytic streptococci being found in profusion on only one occasion.

Case VIII.   Child with extensive burns. Areas on the trunk and right leg were clinically 2nd degree and healed as such. The left arm, which was clinically 3rd degree with a narrow peripheral zone of 2nd degree, was chosen for study. Swabs after cleansing yielded a few colonies of *Staph. albus*. Two biopsies were taken from the central part of the arm. One was 3rd degree, the other severe 2nd degree histologically. The dressings were first removed on the 8th day, when large areas of tough brown slough were seen. Healing appeared to be taking place in the peripheral areas, and this was confirmed by finding epithelium in scrape biopsies. Swabs yielded hæmolytic streptococci and a few colonies of *Staph. aureus*. On the 14th day the wound was examined again. The previously healing peripheral zone had broken down and the whole wound was covered by granulation tissue. A profuse growth of *Staph. aureus* and streptococci was obtained. Local sulphonamide treatment was commenced. Further detailed examination was not made, as no question of 2nd degree type healing appeared possible. It seemed probable that the 2nd degree zone was destroyed by ulceration extending outwards from the infected 3rd degree area.

Case IX.   Girl with extensive flame burns of both lower limbs. After cleansing, no organisms were recovered. On both sides the central areas were of 3rd degree clinically, the peripheral zones of 2nd degree, the latter slightly purpuric and exuding serum. Biopsies were taken from the central areas of both thighs and from the peripheral zones. Histology confirmed the clinical appearances. The right leg was treated with sulphathiazole cream, the left with the jelonet. On the 5th day healing of the peripheral zones of both legs was confirmed by biopsy, which showed a thin epithelial covering on the surface. The central areas were mottled, dry and presumably composed of a thin dry slough. Swabs from all areas gave a profuse growth of coliforms and diphtheroids. By the 12th day the peripheral areas were well healed, but the central 3rd degree areas were covered by sloughs, with thick pus beneath them. Swabs yielded *Staph. aureus* and coliforms. *Staph. aureus* was more profuse on the left side, which had not been treated with sulphathiazole. Healing occurred slowly from the edges of the healed 2nd degree zones over the 3rd degree areas, with the usual scarring.

In this case there was healing of the 2nd degree areas, with typical slow healing of those of 3rd degree, with, however, no apparent re-ulceration of the 2nd degree zone.

Case X.   Child with scalds affecting both feet and left leg, clinically of

HISTOLOGY OF HEALING BURNS

FIG 14—Healing 2nd degree burn (3rd day), taken from the same site as fig 3 Infected with a variety of organisms including *B. coli* and also hæmolytic strepto cocci for 3 days   Healed in 9-10 days   H. and E.   ×90.
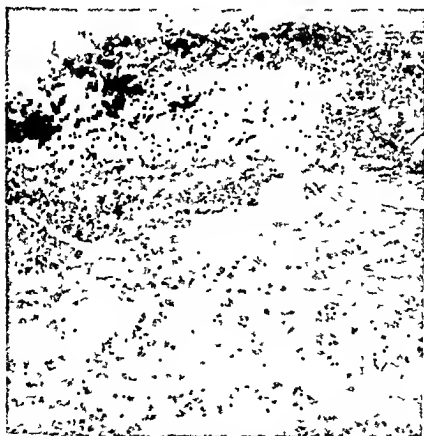
FIG 15—Healing 2nd degree burn (7th day) which showed clinically a severe inflammatory reaction following the appearance of *Staph. aureus* on the 6th day. Note masses of Gram+ cocci on surface of thick purulent exudate. The underlying epithelium, which was in an advanced stage of healing, is not shown. Healing complete by 10th day. Modified Gram.   ×400

varying degree but probably mainly 2nd. The left leg and foot being the more severely affected were observed alone at first; subsequently both feet were studied. A biopsy from the centre of the dorsum of the left foot, probably the most severely affected part, showed a typical 2nd degree appearance. Swabs showed no growth. Treatment was started with oiled silk bags and irrigation with hypochlorite. The treatment therefore differs from that used in the previous cases.

Left leg and foot. On the 2nd day a "flare" was evident round the wound on the dorsum of the foot. On the 4th day *Staph. aureus* was recovered. On the 6th day the child's condition had deteriorated, signs of pulmonary infection appeared and the local inflammatory changes in the foot persisted. Swabs taken from three areas in the wound all grew *Staph. aureus* and hæmolytic streptococci. A biopsy from the wound on the outer side of the leg showed suppuration in and destruction of hair follicles and sweat ducts which had almost certainly survived the original scald. A biopsy from the dorsum of the foot showed exposed corium, with cocci on the surface, and no viable epithelium. There was an absence of the usual covering of fibrinopurulent material found in other cases in this series.

The right foot was examined at the same time and sloughs were found on the sole of the foot. Pus could be squeezed from the open mouths of hair follicles on the dorsum, the surface of which showed little inflammatory reaction and no fibrinopurulent covering; there was no evidence of epithelial regeneration. A swab yielded *Staph. aureus.*

Sulphatbiazole was given by mouth but on the 7th day, because of continued deterioration of the child's condition, treatment was changed to include local sulphanilamide and jelonet. Death occurred on the 9th day. *Post mortem,* there were multiple tiny lung abscesses from which hæmolytic streptococci were recovered. This organism was also recovered from the splenic pulp.

This is the only case in our series in which a proved 2nd degree scald of large area was converted to one of 3rd degree.

DISCUSSION

In spite of the usual precautions in exposing burns for examination, all but two out of fifteen investigated showed the presence of hæmolytic streptococci or *Staphylococcus aureus* at some stage, while in nine both organisms were present. Other associated bacteria included *Staph. albus,* diphtheroids, *B. proteus, Ps. pyocyanea, B. coli* and coarse Gram-negative diplococci.

Bodenham and others have noted such organisms apparently behaving as harmless residents. This we found to be true of genuine 2nd degree burns only, where healing could occur in the presence of hæmolytic streptococci and *Staph. aureus* and without appreciable delay. In 3rd degree burns, on the other hand, pathogenic organisms seldom behaved as innocent residents.

In a typical 2nd degree burn epithelial regeneration was evident to the naked eye within 48 hours. The surface, when not unduly obscured by exudate, showed numerous slightly raised small dots of pin-head size, probably around hair follicles. By the fourth or fifth day the epithelial covering was continuous. It dried on exposure to air and had the appearance of a thin varnish. At first the colour was red to pink, but during succeeding days the area became paler

and more opaque, approaching the tint and appearance of normal skin. Naturally it was difficult to say exactly when complete healing had occurred, but in general a 2nd degree burn could be considered as clinically healed in 7-12 days, so that further dressings were unnecessary.

Great variations were observed in the character of the inflammatory response. In the protocols we frequently refer to the presence of a "flare". This measured up to 1 cm. in breadth and affected the margin of the burn as well as the adjacent unburnt skin. This "flare" lasted for 24-48 hours and was seen in approximately 60 per cent. of the cases. It may well be that this is what others have called burn erysipelas and possibly it represents a limited lymphangitis with congestion and œdema: we did not investigate it histologically.

Another interesting response was that which occurred on the surface of healing 2nd degree burns which had become infected with hæmolytic streptococci or *Staph. aureus*. The surface exudate which formed gave the impression that the new-formed epithelium was being destroyed. From the limited biopsy material obtained from such sites, however, we found that the underlying epithelium was still intact and that the effect of the exudation from the vessels of the dermis was to remove the organisms as far as possible from the proliferating epithelium. The latest time at which we observed this inflammatory reaction was the 7th day. On the other hand we not infrequently recovered *Staph. aureus* from almost healed 2nd degree burns which in themselves showed little or no reaction, though pustules occurred in the hair follicles of surrounding unburnt skin. In heavily infected 3rd degree burns the inflammatory reaction on occasion spread directly to unburnt skin, resulting in spreading ulceration of varying severity. This could occur also where 3rd degree areas were surrounded by 2nd degree zones, bringing about the conversion of a 2nd degree zone to that of a 3rd degree.

Summary

1. A small series of 2nd and early 3rd degree burns has been examined in detail, using the method of biopsy to assess the extent of the initial injury and to determine the mode of healing.

2. Epithelialisation of 2nd degree burns takes place multifocally from the viable epithelial cells lining sweat ducts and hair root follicles. There appears to be no latent period in this process.

3. In pure 2nd degree burns healing can occur in the presence of hæmolytic streptococci and *Staphylococcus aureus*, singly or together, with little if any retardation.

4. In mixed 2nd and 3rd degree burns, if infection becomes established in 3rd degree areas, healing of neighbouring 2nd degree areas is frequently delayed.

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AN EXPERIMENTAL STUDY OF THE HEALING OF WOUNDS, WITH SPECIAL REFERENCE TO THE ACTION OF HEART EXTRACT POWDER (DOLJANSKI) *

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(PLATES VIII AND IX)

An indolent wound is always a vexatious problem, but in times of war it acquires an immediate importance to be measured only by the wastage of man power for which it is responsible. Inevitably, therefore, the problem is tackled with a new zest when the shooting begins—and it is not an easy problem to tackle. In recent articles Kerr and Werner (1944-45) and Werner (1944-45) have reported a series of eighty indolent wounds, burns or ulcers which they had treated by the local application of a certain heart extract powder (H.E.P.) prepared for them by Doljanski and his colleagues (Werner and Doljanski, 1942). Their results were conspicuously successful, being marred by only seven failures. Such success is important enough in itself as a therapeutic achievement, but it possesses far wider biological implications if Kerr and Werner are right in their conclusion that H.E.P. promotes the healing of indolent wounds in virtue of its capacity to promote the growth of fibroblasts and epithelial cells *in vitro.*

The issues, both scientific and practical, are so important that we have carried out a series of experiments in rabbits and guinea-pigs, designed, so far as possible, to test the biological effects of H.E.P. in relation to the processes of healing of healthy wounds.

TECHNICAL PROCEDURES

The experimental wounds

These were cut, under nembutal and other anæsthesia, to a gentian violet circular template 17·5 mm. in diameter, with as much uniformity as possible

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in respect of area, depth, distribution and every other technical detail, and always with sterilised instruments. The backs of the animals were closely clipped with scissors, the skin " sterilised " with 1 per cent. parachlormetaxylenol in 50 per cent. spirit and painted generously with " Portex plastic skin " to minimise the risk of infection. Bleeding was usually moderate or slight. The wounds were paired symmetrically (fig. 1), and the site of the experimental and control wounds rotated from one animal to the next in every experiment, because rabbit wounds on the anterior half of the back heal more rapidly than those on the posterior half. Charts of the wounds were prepared from cellophane tracings as described by Young et al. (1941). In the statistical analysis, the average of two measurements taken on the 2nd and 3rd days after wounding was used. In every experiment the mean size of wound was in close agreement, although there was always a fairly large scatter. Septic and non-healing wounds were omitted. For wounds that regressed, the period until final closure was taken as the length of time to heal.

Fig. 1.—Distribution of wounds to scale.

There is one important respect in which our experimental procedure departed from the technique enjoined by Kerr and Werner in their treatment of indolent wounds in man, namely daily or alternate daily dressing. We have dusted our wounds twice only during the "latent phase", as follows. When all bleeding had been arrested the wounds were dusted liberally with heart extract powder (vide infra) or embryo extract powder (vide infra) as the case might be—approximately 25 mg. of powder to each wound (mean area 300 sq. mm. or thereby). The wounds were then allowed to dry without any dressing, and to form a scab. Forty-eight hours later the edges of all wounds, including the untreated controls, were broken by pulling on the adjacent skin, and the experimental wounds were again dusted as before, each with its appropriate powder. Thenceforth, healing was allowed to proceed under a reconstituted scab without further interference. In our opinion there are four sound reasons for this modification of Kerr and Werner's technique, having regard to the aims of our investigation : (1) our experimental wounds are healthy wounds possessing a natural disposition to heal ; they do not need a daily or alternate daily stimulus to mend as indolent wounds are said to do ; (2) H.E.P., applied as we have described and incorporated in the primary scab, persists in the floor of the wound for upwards of twenty-five days ; (3) daily moist dressing of animal wounds causes a steep rise in the incidence of sepsis ; and (4) it is useless to apply the powder to the surface of a dry wound which is already covered with a scab.

Heart extract powder (H.E.P.) and embryo extract powder (E.E.P.)

If it had been possible, we should have preferred to test a heart extract powder prepared by Doljanski and his colleagues and certified to be biologically active in promoting cell growth *in vitro*, such as Kerr and Werner used in their successful treatment of indolent wounds in man, but we were disappointed in our hopes of obtaining this. A certain large sample of Doljanski's own H.E.P. was sent to this country early in 1944 to be tested experimentally on healthy wounds and clinically on indolent wounds, but it was almost immediately declared by him to be inert—presumably on the basis of in-vitro tests. Therefore, our experiments have been made with a heart extract powder kindly prepared for us by Dr J. N. Davidson in strict accordance with Doljanski's written instructions (private communication, 7.2.1944). We are authorised by Dr Davidson to say that his H.E.P. (sheep) possesses very poor growth-promoting capacity *in vitro*, whereas material prepared by him in the same

way from sheep embryo pulp (E.E.P.) possesses this capacity in very considerable measure (Davidson and Waymouth, 1945). These two powders were prepared from adult sheep heart and whole sheep embryo respectively, in exactly the same way, namely by extracting the minced tissue with saline solution, adding alcohol to the extract and collecting and drying the precipitate so formed.

Dr Davidson provided two samples of H.E.P., " A " and " B ". Sample " A " (like the whole sheep embryo extract) was prepared by precipitating a saline extract of adult sheep heart with four volumes of 96 per cent. alcohol in accordance with Doljanski's instructions; the effects of this sample were matched with those of E.E.P. on healthy wounds in the first experiment of our series and the progress of healing of healthy wounds, some treated with H.E.P., others with E.E.P., was matched with that of untreated control wounds in our second and third experiments. In all the subsequent experiments, sample " B " was used ; it was prepared by precipitating a saline extract of adult sheep heart with two parts of 96 per cent. alcohol (Kerr and Werner). There seems to be no significant difference between the two extracts, " A " and " B ", either chemically or biologically, and none has been suggested at any time by Doljanski and his colleagues. Both samples of H.E.P., which were kept in the ice-chest when not in use, were tested bacteriologically at intervals and proved to be sterile on all occasions.

EXPERIMENTAL OBSERVATIONS

Expt. 1. Two rabbits only, flemish giants of same strain, ×6 wounds each : 3 treated with H.E.P., 3 with E.E.P.

Result. This pilot experiment revealed no significant difference in the effect of H.E.P. and E.E.P. on the rate of healing of healthy wounds. A slight disparity in the rate of healing up to the 20th day, with the advantage to the E.E.P. in both rabbits, seemed to warrant the making of experiments with H.E.P. and E.E.P. side by side in the same rabbit, together with untreated controls, discounting any minor constitutional effects of the powdered extracts.

Expt. 2. Twelve rabbits, all chinchillas of same strain, ×6 wounds each : 2 treated with H.E.P., 2 with E.E.P. ; 2 untreated controls. One rabbit died 10 minutes after wounding.
Object. To compare the effects of H.E.P. and E.E.P. on the rate of healing of healthy wounds in the presence of untreated control wounds.
Expt. 3. Twelve rabbits, all flemish giants of same strain, ×6 wounds each : 2 treated with H.E.P., 2 with E.E.P. ; 2 untreated controls. Two rabbits died shortly after wounding.
Object. Repetition of expt. 2.

The results of these two experiments are summarised in table I.

Summary of conclusions (expts. 2 and 3 combined)

H.E.P. has a definite retarding effect on the rate of healing of healthy wounds unless, perhaps, it exercises a constitutional stimulating effect on the healing of other wounds in the same animal, which is more potent than its local stimulating effect when applied directly to a wound or wounds (Auerbach and Doljanski, 1945 : vide expt. 4 *infra*).

The wounds treated with E.E.P. took longer to heal, on the average, than the untreated controls, but for expts. 2 and 3 separately the difference was below the level of significance. If the two experiments are combined the difference is statistically significant.

TABLE I

Local effects of heart extract powder and embryo extract powder on rate of healing of wounds

	Experiment 2			Experiment 3		
	H.E.P.	E.E.P.	Untreated controls	H.E.P.	E.E.P.	Untreated controls
No. of wounds	22	22	22	20	20	20
Mean area (sq. mm.)	294	293	301	323	328	325
Standard deviation	38·8	43·2	49·3	54·2	48·3	57·1
Healing of wounds						
No. of wounds	21	22	21	18	17	17
Mean number of days to heal	28·33	26·86	24·24	28·89	28·18	26·47
	Diff.	t	P	Diff.	t	P
H.E.P.-controls	4·09	3·590	<·01	2·42	2·107	·05>P>·02
E.E.P.-controls	2·62	1·661	·1>P>·05	1·71	1·442	·2 >P>·1
H.E.P.-E.E.P.	1·47	0·861	·4>P>·3	0·71	0·713	·5 >P>·4

The average healing time was slightly but not significantly greater for H.E.P. than for E.E.P.

There is no indication that an agent such as E.E.P., which has been proved to promote actively the growth of fibroblasts and other cells *in vitro*, will *ipso facto* stimulate the growth of fibroblasts and other cells *in vivo* in such a manner as to accelerate the processes of healing of a healthy wound. In fact, within the limits of our experimental procedures, a healthy wound heals more promptly when it is not treated with such a substance.

Expt. 4. Twenty rabbits, all chinchillas of same strain, namely 5 rabbits ×6 wounds, all treated with H.E.P.; 5 rabbits ×6 wounds, all untreated; 10 rabbits ×6 wounds, 3 treated with H.E.P. and 3 untreated.

Object. To test whether H.E.P. locally applied to three wounds exercises any constitutional effect on the rate of healing of three other untreated wounds in the same animal. The progress of healing of the wounds according to their treatment is shown in table II.

The untreated wounds had for each set a significantly lower average time of healing than the wounds treated with H.E.P. Hence, expt. 4 confirms the finding of expts. 2 and 3, namely that the local application of H.E.P. retards the healing of healthy wounds.

Table III shows that when expts. 1-4 are combined, the differences are again significant.

TABLE II

Constitutional and local effects of heart extract powder on rate of healing of wounds

	5 rabbits ×6 wounds all treated with H E P	10 rabbits ×6 wounds of which 3 were treated with H E P and 3 untreated		5 rabbits ×6 wounds all untreated
	H E P controls	H F P experimental	Untreated experimental	Untreated controls
No of wounds	30	30	30	30
Mean area (sq mm)	300	311	316	289
Standard deviation	55 5	46 7	59	66 3

Healing of wounds

No of wounds	30	25	27	28
Mean number of days to heal	28 73±0 62	30 76±0 96	25 22±0 78	26 25±0 65

1 *Constitutional effect of H E P on rate of healing*

	Difference
Untreated controls untreated experimental	1 03±1 01
H E P experimental H E P controls	2 03±1 14

Neither of these two differences is significant   Hence it follows that H E P exercises no constitutional effect on the rate of healing of healthy wounds

2 *Local effect of H E P on rate of healing*

	Difference
H E P controls untreated controls	2 48±0 90
H E P experimental untreated experimental	5 54±1 24
All rabbits Wounds treated with H E P untreated wounds	3 91±0 76

Three of the five unhealed wounds in the H E P experimental series and one of the two unhealed wounds in the untreated control series developed abscesses   The arrest of healing of the remaining six wounds could not be satisfactorily explained infection was suspected but bacteriological examination was inconclusive

TABLE III

Local effect of heart extract powder and embryo extract powder on rate of healing of wounds

Expts 1, 2, 3 and 4 combined

Treatment	No of wounds	Mean time of healing in days	Standard deviation
H E P	100	29 21±0 40	3 07
E E P	45	27 36±0 75	5 02
Untreated	93	25 54±0 38	3 60

Differences

H E P untreated 3 67±0 55
E E P untreated 1 82±0 84
H E P E E P 1 85±0 85

Expt. 5. Six white guinea-pigs of same strain: 3×2 primary wounds treated with H.E.P. ("sensitised"); 3×2 primary wounds untreated ("non-sensitised"); all $\times 2$ secondary wounds after an interval of 13 days: all secondary wounds treated with H.E.P.

Object. To test whether H.E.P. applied locally to wounds is anaphylactogenic.

Theoretical principles of experiment. This experiment does not conform to the classical design for the study of the phenomena of anaphylaxis, but it is a legitimate adaptation of that design to test a suggestion by Werner (p. 519), that H.E.P. applied locally to human wounds is anaphylactogenic " so that the individual treated responded with a form of anaphylactic shock ". The guinea-pig was chosen as the experimental animal on account of its sensitivity to anaphylactogens. The ratio of the area of our experimental wounds to the total surface area of the guinea-pig was approximately 1 : 80, *i.e.* roughly equivalent to a wound or wounds amounting to 38 sq. inches in an average man. Accordingly, it might reasonably be expected that, even if H.E.P. were only feebly anaphylactogenic, some visible reaction should be apparent in and around a second generation of wounds made at an interval of 13 or 14 days after the primary wounds if both primary and secondary wounds were liberally dusted with H.E.P. Conceivably this reaction might be expressed by an acceleration of the rate of healing of the secondary wounds.

Result. In this pilot experiment, the difference between the mean healing time of the primary wounds of the " sensitised " group (treated with H.E.P.) and those of the " non-sensitised " group (untreated) was significantly in favour of the untreated wounds, (difference $= 8 \cdot 5$ days; $t = 2 \cdot 996$; $\cdot 02 > P > \cdot 01$). Thus H.E.P. seems to retard the processes of healing of healthy wounds in guinea-pigs as well as in rabbits.

The difference between the mean healing time of the secondary wounds of the two groups (both treated with H.E.P.) was no greater than would be expected from mere chance (difference $= 1 \cdot 3$ days; $t = 0 \cdot 612$; $\cdot 6 > P > \cdot 5$). Hence, this small experiment would suggest that previous " sensitisation " of a guinea-pig with H.E.P. has no effect on the rate of healing of a healthy wound.

Expt. 6. 24 guinea-pigs of same (M.R.C.) strain: 8×2 primary wounds treated with H.E.P. ("sensitised"); 8×2 primary wounds untreated ("non-sensitised"); 8×2 primary wounds untreated (control); all $\times 2$ secondary wounds after interval of 14 days. Secondary wounds of "sensitised" and "non-sensitised" groups treated with H.E.P.; secondary wounds of control group untreated.

Object. Repetition of expt. 5 with addition of untreated control series.

Results. These are shown in table IV.

Summary of observations and conclusions (expts. 5 and 6)

1. None of the guinea-pigs " sensitised " by a liberal application of H.E.P. to paired primary wounds died when the " assaulting " dose of H.E.P. was applied 13 or 14 days later to paired secondary wounds.

2 Neither erythema nor any other manifestation of tissue hyper sensitiveness, *e g* increased exudation of plasma with thickening of the scab, was apparent in or around the margins of the secondary wounds following the application of the " assaulting " dose of H E P.

TABLE IV

Effects of heart extract powder on rate of healing of primary and secondary wounds in guinea pigs

	8 sensitised guinea pigs			8 non sensitised guinea pigs			8 untreated controls		
No of wounds Treatment Mean area (sq mm) S.D	16 prim H E P 300 54	14 days	16 sec H.E.P 355 65	16 prim None 301 57	14 days	16 sec H E P 352 35	16 prim None 301 44	14 days	16 sec None 350 40
Healing of wounds									
No of wounds Mean number of days to heal	16 24 6		13 27 3	16 19 9		16 24 8	16 20 3		16 23 6
Primary secondary	Diff 2 7 t 1 618 P 2>P> 1			Diff 4 9 t 5 178 P < 01			Diff 3 3 t 2 159 P 05>P>02		

		Primary wounds			Secondary wounds		
		Diff	t	P	Diff	t	P
' Sensitised '	" non sensitised '	4 7	4 066	< 01	2 5	1 741	1>P> 05
Sensitised	controls	4 3	3 824	< 01	3 7	1 820	1>P> 05

A hitch occurred owing to withdrawal of sugar-beet pulp by "zoning" of foodstuffs and substitution of bran mash on 14th day of healing of secondary wounds all animals lost weight up to 15 per cent (sensitised " group to least extent) before adjustment to new diet There was no evidence of sepsis in any of the wounds

3 The rate of healing of the secondary wounds in the " sensitised " group was not accelerated , on the contrary, it was slightly but not significantly retarded as compared with the secondary wounds in the " non sensitised " and control groups

4 These three observations preclude any suggestion that H E P applied locally to a relatively large wounded area, amounting approximately to 1/80th of the body surface is anaphylactogenic in the guinea pig , taken in conjunction with the results of expt 4 (rabbit), they would indicate that H E P exercises no constitutional effect whatsoever in so far as the healing of healthy wounds is concerned

5 The results of expts 5 and 6 with the guinea-pig reaffirm the conclusion reached in expts 2 4 with the rabbit that the local application of H E P to a healthy wound consistently retards the normal processes of healing

*Histological observations on the effects of heart extract powder and
other agents on the healing of experimental wounds in the rabbit*

The unfailing consistency with which H.E.P. retarded the
processes of healing of healthy wounds in the rabbit and guinea-pig
seemed to predicate some alteration of tissue reaction which might
be demonstrable microscopically. Hence it was decided to follow
histologically the progress of healing of wounds treated with H.E.P.

Expt. 7. Five rabbits, all chinchillas of the same strain, ×6 wounds;
3 treated as in previous experiments with H.E.P. and 3 untreated, rotating
from rabbit to rabbit : rabbits killed in series on 5th, 10th, 15th, 20th and
25th days for histological sampling.

Results. H.E.P. evokes a foreign-body giant cell reaction. This
begins to manifest itself before the 5th day of experiment by a
congregation of granulocytes and histiocytes around the particles of
H.E.P., accompanied by a proliferation of fibrocytes. During the
next ten days the foreign-body giant cells gradually take shape
from the histiocytes immediately investing the particles, and by
the 20th day they are conspicuous. Their intimate relationship to
the H.E.P. particles is further emphasised by the fact that they can
be seen " nibbling " the particles in precisely the same manner in
which osteoclasts " nibble " laminæ of bone during lacunar absorption
(fig. 2).

Normally, the healing of a healthy experimental wound proceeds
from beginning to end without trace of foreign-body giant cell reaction.
Hence, it seems reasonable to deduce that H.E.P. retards the normal
processes of healing of healthy experimental wounds by setting in
motion an abnormal proliferative mechanism in which granulocytes,
histiocytes, foreign-body giant cells and fibrocytes all participate.

Hitherto, arising out of our experience of the retardation of healing
of healthy animal wounds treated with H.E.P., we had been more
and more perplexed by the conspicuous success achieved by Kerr
and Werner in the treatment of indolent wounds in man by H.E.P.—
assuming that their success was directly attributable to H.E.P. and
not to any other factor. We are well aware that the gulf between
an indolent wound in man and a healthy wound in a rabbit or guinea-
pig is wide—so wide that it probably cannot be bridged by any
experimental technique as yet devised. Nevertheless, when the
inertia of an indolent wound is overcome, the cellular elements of
repair are the same as in a healthy one, so that the ultimate problem
of healing is likely to be indivisible. Therefore the histological dis-
closure of an abnormal mechanism affecting histiocytes and fibrocytes
in particular, actuated by H.E.P., suggested a possible explanation of
the paradox that an agent which had been proved to retard the
healing of healthy wounds might still initiate the healing of an
indolent wound—provided that it were able to set the same abnormal

STIMULATION OF WOUND HEALING

FIG 2.—"Lacunar absorption" of H E P particles   25th day   ×150

FIG 3.—Sudan III particles dissolved in preparation of paraffin section, leaving spaces surrounded by giant cells   20th day   ×150

mechanism in motion in an indolent wound in man as it does in a healthy wound in a rabbit. This question should readily be decided by a series of biopsies of indolent wounds in man before and after treatment with H.E.P. Meanwhile it can be approached from another angle, because H.E.P. is not the first substance to enjoy a vogue in the healing of indolent wounds in man. Scharlach R had a similar vogue about thirty years ago. It was introduced for this purpose by Schmieden (1908) as "Scharlachsalbe"—a saturated (8 per cent.) solution of Scharlach R (Grübler), *i.e.* toluol-azotoluolazo-β-naphthol, solvent not stated. It was applied rather indiscriminately, it would appear, by many surgeons in Germany, Britain and elsewhere, often with the substitution of Sudan III (benzol-azobenzolazo-β-naphthol) for Scharlach R, and was claimed to be highly effective in promoting the healing of indolent wounds in man and in improving the " take " of skin grafts (Krajča, 1908).

Expt. 8. Three rabbits, all flemish giants of the same strain, ×6 wounds ; 2 treated with H.E.P., 2 with Sudan III (C.I. no. 248 as supplied by G. T. Gurr) and 2 with kaolin ; killed in series on the 10th, 15th and 20th days.

Expt. 9. Six rabbits, all flemish giants of same strain, 5×6 wounds ; 2 treated with Sudan III, 2 with Scharlach R (C.I. no. 258 as supplied by G. T. Gurr) and 2 untreated ; killed in series on 5th, 10th, 20th and 25th days : 1×6 wounds, all untreated, killed on 20th day.

Object. To compare the histological effects of H.E.P., Sudan III and Scharlsch R on the healing of healthy wounds in the presence of untreated control wounds in the same animal.

Results. Sudan III and Scharlach R both evoke a foreign-body giant cell reaction (fig. 3) like H.E.P. ; the evolution of the giant cells proceeds *pari passu* with all three substances. H.E.P. evokes a moderate papillary hyperplasia of the reparative epithelium (fig. 4), Sudan III a more vigorous reaction of the same kind (fig. 5), Scharlach R an exuberant but benign epithelial hyperplasia (fig. 6) as originally described by Fischer (1906).

Wounds treated with Sudan III and Scharlach R tend to lag behind untreated control wounds in the same animal just as with H.E.P.

COMMENTARY

Expressed briefly, four essential principles have been adduced by Doljanski and his colleagues to explain the alleged remarkable efficacy of H.E.P. in the healing of normal wounds in rats as well as of indolent wounds in man.

1. "Extracts of certain adult organs—heart, smooth muscle and brain—activate . . . the growth of fibroblast colonies *in vitro* to a greater extent than embryonic extract of the same concentration " (Auerbach and Doljanski, 1944, p. 38). This claim is diametrically opposed to the findings of Trowell and Willmer (1939). So far as H.E.P. itself is concerned, Davidson and Waymouth have reported

that it was inferior to powders prepared in the same way from embryo sheep heart and whole sheep embryo (E.E.P.) in five tests out of six; it appeared to be more active in only one instance.

2. The second principle re-states a suggestion first made by Carrel (1921 *et ante*) that substances which promote the growth of fibroblasts and other cells *in vitro* do, *ipso facto*, promote the growth of fibroblasts and other cells *in vivo*, as in a healing wound (Kerr and Werner). The results of our expts. 2 and 3 would indicate that there is no direct correlation between the growth-promoting activity of a substance *in vitro* and *in vivo* inasmuch as a particular substance (E.E.P.), which was proved to possess very considerable growth-promoting activity *in vitro*, did not accelerate the healing of healthy wounds in the rabbit; on the contrary, it retarded it. H.E.P., possessing very poor growth-promoting activity *in vitro*, retarded the healing of similar wounds to a greater extent than E.E.P.

3. The third principle rests on a suggestion by Werner that H.E.P is anaphylactogenic in man. Our expts. 5 and 6 revealed no trace of anaphylactogenic activity in guinea-pigs. The only effect which we have been able to identify was a retardation of the healing of healthy wounds in the guinea-pig just as in the rabbit.

4. The fourth principle, recently elaborated by Auerbach and Doljanski (1945) "envisages the possibility" that the stimulating effect of H.E.P. on wound healing is "not local but of a general nature"; that is to say, H.E.P. functions as a "wound hormone" (Haberlandt, 1921) in a reasonably strict sense of the term. There are several strange anomalies in Auerbach and Doljanski's experimental data but we shall mention only one. H.E.P. is only slightly soluble in normal saline or Tyrode. Nevertheless, when it was applied externally in an ointment (lanolin anhydrous) to single wounds of 20 mm. diameter in rats, eight to ten times on alternate days, it produced a greater general effect in accelerating the healing of untreated wounds in the same animals than 8-10 intraperitoneal injections of 1 c.c. of 33 per cent. saline extract of adult chicken heart, *i.e.* the saline extract from which the slightly soluble H.E.P. was prepared by precipitation with alcohol.

The results of our expt. 4 would indicate that H.E.P. applied locally to three healthy wounds exercises no general effect whatsoever on the rate of healing of three other healthy (untreated) wounds in the same rabbits, while it retards the healing of those wounds to which it is directly applied.

In the upshot, we are totally unable to reconcile our results with the second, third and fourth principles adduced by Doljanski and his colleagues, and their first principle has already been challenged by experts in tissue culture technique.

Nevertheless, the salient fact remains that 73 of Kerr and Werner's series of indolent wounds healed, and sometimes speedily, under treatment with H.E.P.. while only 7 failed to heal. At least 63 of

STIMULATION OF WOUND HEALING

Fig. 4.—H.E.P. Papillary hyperplasia of reparative epithelium at margin of healing wound : 20th day. ×60.

Fig. 5.—Sudan III. Papillary hyperplasia of reparative epithelium, enveloping particle of Sudan III. 20th day. ×60.
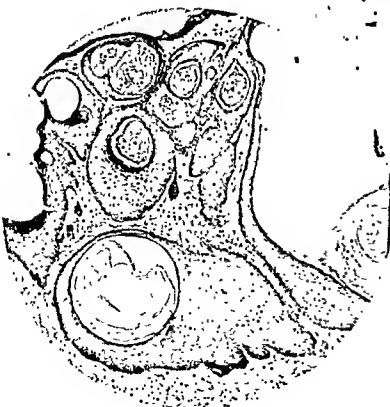
Fig. 6.—Scharlach R. Papillary hyperplasia of reparative epithelium; 25th day. ×60.

their patients (and possibly more) had been treated more or less intensively with sulphanilamide for prolonged but variable periods up to 270 days, and one (D.L. aged 27) would appear to have been suffering from sulphonamide poisoning at the time when sulphanilamide was stopped and treatment with H.E.P. began. Mitchell (1944, p. 229) says that " sulphanilamide and vaseline gauze must be one of the most misused materials in the whole realm of surgery, and one has seen cases with indolent ulceration and pale granulations where this dressing has been used continuously over periods of two or three months ". It is most regrettable, therefore, that Kerr and Werner were unable to include a series of " sulphonamide controls " in their clinical experiment. Without strict control of antecedent treatment, an animal experiment comprising any number of wounds would rightly be regarded as null and void. In these circumstances, the claims of Scharlach R and Sudan III are certainly not inferior to those of H.E.P. in respect of their ability to promote the healing of indolent wounds and the " taking " of skin grafts in man because they had their vogue before the issue was complicated by chemotherapy.

If it is really true—and we cannot tell—that these totally dissimilar substances, a denatured protein and two fat-soluble azo dyes, can each promote the healing of indolent wounds in man, it becomes a reasonable working hypothesis that the peculiar biological reaction manifested to all three alike, namely a foreign-body giant cell reaction, is more significant than the nature of the reagent—and there is a wide variety of other agents, already known, which can evoke a foreign-body giant cell reaction in animal tissues.

In conclusion, we have been informed through official channels that at least three long-suffering patients with indolent wounds have been treated in the Middle East with intramuscular injections of a certain water-soluble fraction of H.E.P. The rationale of this treatment is said to be a " logical " outcome of experimental and clinical observations by Doljanski *et al.*, and by Kerr and Werner respectively. We are not impressed by the " logic ". Indeed, on the evidence available, we would submit that an equally good—or perhaps a better—plea could be sustained for the parenteral injection of one or other of the azo dyes in the treatment of indolent wounds in man, even though one of us (Young, 1928) has shown that Sudan III injected parenterally in high dosage can determine an acute cytolytic necrosis of the liver of the rabbit.

SUMMARY

The progress of healing has been followed in 462 experimental wounds in rabbits and guinea-pigs.

Heart extract powder (adult sheep heart) and embryo extract powder (whole sheep embryo) applied directly to the surface of healthy wounds consistently retard the healing process.

There is no direct correlation between growth-promoting activity *in vitro* and *in vivo* in so far as the healing of healthy wounds can be accepted as a criterion.

H.E.P. exercises no general or constitutional effect on the rate of healing of healthy wounds, it is not anaphylactogenic in the experimental conditions cited and it does not influence either the onset or the progress of infection.

Applied directly to the surface of a wound, H.E.P. evokes a nonspecific foreign-body giant cell reaction similar to that evoked by two azo dyes—Scharlach R and Sudan III—which enjoyed a vogue some thirty years ago as stimulants to the healing of indolent wounds.

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THE DIFFUSION OF ANTISEPTICS THROUGH AGAR GELS, WITH SPECIAL REFERENCE TO THE AGAR CUP ASSAY METHOD OF ESTIMATING THE ACTIVITY OF PENICILLIN

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Since the Oxford workers (Abraham *et al.*, 1941) suggested the agar cup assay method as a standard for the estimation of penicillin, great use of this method has been made. Many modifications have been suggested (McKee *et al.*, 1944; Foster and Woodruff, 1943, 1944; Schmidt and Moyer, 1944) and many details of technique have been found to influence the result. Attempts have also been made to apply the same technique to the evaluation of the action of other antiseptics, though it is evident that the size of the ring of inhibition of growth depends primarily not only on the antiseptic activity but also on the rate of diffusion of the antiseptic through the agar (Tobie and Ayres, 1944).

The original published curve giving the relationship between the size of the ring and the concentration of penicillin placed in the cup is obviously not a simple one (Abraham *et al.*). The curves published by Schmidt and Moyer showing the effect of pre-incubation and pre-refrigeration emphasise still further the complicated nature of the relationship. On the other hand the rates of diffusion of proteins, dyes, etc., through water and gels have been investigated and theoretical formulæ used with considerable success in recent years. A valuable review of the work done since the publication of Svedberg's monograph, " The ultracentrifuge ", has been made by Neurath (1942).

In order to determine if the diffusion of antiseptics through nutrient agar could be represented by such theoretical formulæ and how this diffusion and the antiseptic action combined to produce the relationships found in the agar cup assay method, we have chosen first to examine its application to a coloured antiseptic. By this choice we have been able to follow diffusion because of the colour and to relate this with the zone of inhibition due to antiseptic action in a way that is not possible with colourless penicillin. Having thus found a theoretical formula whose constants we are able to measure, we

have applied analogous formulæ to curves for penicillin. By choosing suitable constants for penicillin we have been able to calculate curves which fit within the limits of experimental error. The applicability of such formulæ explains the influence of some of the most important technical factors which modify the experimental results.

In a paper entitled " The diffusion coefficients of molecules and ions from measurements of undisturbed diffusion in a stationary medium " Eversole and Doughty (1935) give the formula for the diffusion of neutral particles of a substance with a concentration m_0 placed in a cylindrical tube adjacent to a column of pure water. If the concentration at a distance x from the junction of the solution and the water after a time t is m', and the diffusion coefficient of the solute is given by D, the relationship is given by :—

$$m' = m_0 \, e \, \frac{-x^2}{4Dt} \text{ or } ln \ m_0 - ln \ m' = \frac{1}{4D} \frac{x^2}{t} \qquad . \qquad . \qquad . \quad (1)$$

D is the constant in the well known differential equation $\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2}$, which defines D as the amount of solute which would diffuse across unit area under a concentration gradient of unity in unit time if the rate were constant during that time (Lewis, 1918). For spherical molecules of radius r in a solvent of viscosity η it is given by $D = \frac{RT}{6\pi\eta Nr}$ where R is the gas constant, N is Avogadro's number and T the absolute temperature.

It follows that D varies with the absolute temperature and viscosity according to the equation

$$\frac{D_{t_1}}{D_{t_2}} = \frac{T_1}{T_2} \frac{\eta_2}{\eta_1} \qquad . \qquad . \qquad . \qquad . \qquad . \quad (2)$$

For the motion of charged particles a correction for the influence of potential gradient is given, but Lehner and Smith (1936) point out that in the case of dye ions diffusing into water the presence of a few equivalents of sodium chloride in solution prevents the development of a potential gradient. The formula for neutral particles can thus be applied even to ionised dyes.

Nutrient agar media usually contain $\frac{1}{2}$-1 per cent. of salt and, as the dilution of the antiseptic causing inhibition of growth is much below this, the conditions of assay would suggest that formula (1) should be applicable. The solute is of course an agar gel instead of pure water, but although the value of D will be expected to be considerably reduced by increasing concentrations of agar, the formula can still be applied. The antiseptic must not of course be precipitated by any constituent of the medium.

Crystal violet

Formula (1) may be rewritten with logarithms to the base ten as :—

$$4D \; 2 \cdot 30 \; (\log m_0 - \log m') = \frac{x^2}{t}.$$

We decided to test its applicability to a solution of crystal violet diffusing into $2\frac{1}{2}$ per cent. agar containing meat extract, 1 per cent. peptone and $\frac{1}{2}$ per cent. salt adjusted to pH $7 \cdot 6$—our stock nutrient medium. The results at room temperature are shown in fig. 1, obtained by placing a few ml. of 1 : 500 crystal violet on the top of
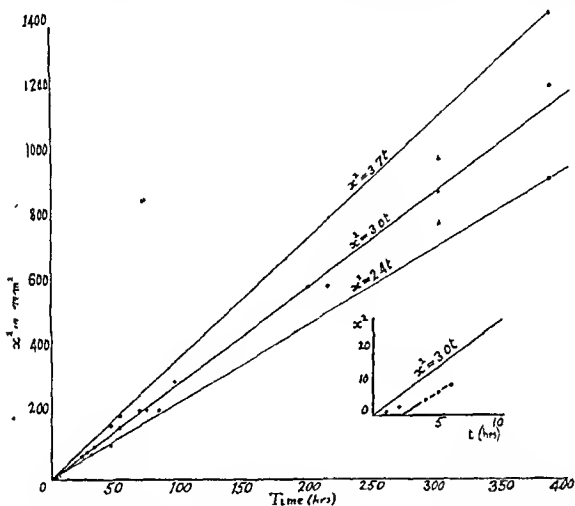
FIG. 1.—Diffusion of crystal violet in $2\frac{1}{2}$ per cent. nutrient agar in cylindrical tubes at 15° C.

$$m_0 = 2 \times 10^{-3} \qquad\qquad m' = 2 \times 10^{-6}$$
$$D = 0 \cdot 109 \text{ mm.}^2/\text{hr.} \qquad \log m_0 - \log m' = \frac{1}{4D \; 2 \cdot 30} \cdot \frac{x^2}{t}$$

cylindrical tubes of the medium and measuring the rate of movement of the coloured edge corresponding to a tube containing 1 : 500,000 crystal violet. Log m_0—Log $m' = 3$ and x^2/t is a straight line which gives $D = 3 \cdot 0 \times 10^{-7}$ cm.^{2}/sec. $= 0 \cdot 109$ mm.^{2}/hr. The experimental results agree well with this except for a slight lag during the first few hours, probably due to the movement of salts and water between the solution and the agar. The exact value of x becomes more difficult to determine when it becomes large, but by reading a value of x definitely above and another below the 1 : 500,000 value of m' and averaging the result it is still possible to make reasonably accurate readings even after 14 days (fig. 1).

Can these results be applied to circular plates as well as cylindrical tubes ? The distance x will correspond to the distance from the edge of the cup to the edge of the coloured ring. (The diameter of the ring, which is the value plotted in most published papers, equals $2x+d$, where $d = $ diameter of the cup.) The dye, instead of diffusing out through a constant cross-sectional area, will be spreading through an increasing area proportional to the circumference of the ring. This will affect not only the concentration at each point but the concentration gradient. During the early hours of diffusion the concentration gradient at the edge of the ring is very steep—several hundred-fold dilution in a few mm.—and the correction appears to be of no practical importance. We have followed the coloured edge of
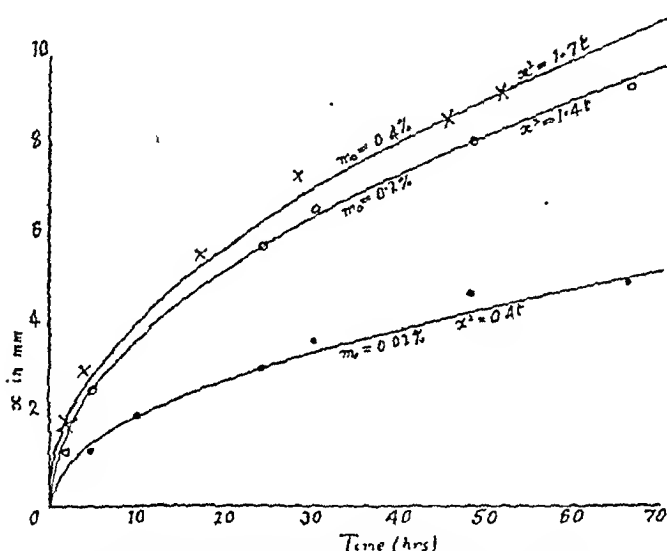
FIG. 2.—Diffusion of crystal violet in agar plates at 15° C.
Concentration at edge of visible colour $= m' = 8 \times 10^{-5}$
$D = 0.109$ mm.^{2}/hr.

the ring of diffusing dye and plotted x^2/t for up to two days without detecting any certain deviation from the straight line. After this period the colour in normal thin agar plates becomes too diffuse to allow the position of the edge to be read accurately. (The value m' is given by the least concentration of crystal violet visible in a plate of the thickness used in this case.) Fig. 2 shows the variation of x with t for three different concentrations in the cup, using the same value of D as found by the cylindrical tube method.

Having determined the applicability of the formula to the diffusion of the dye from the cup to the agar in a plate, the dye concentration at any distance from the cup at any time can be calculated for any concentration in the cup. What is the concentration time relationship at the edge of the zone of inhibition of *Staphylococcus aureus* by crystal violet ? Beyond this, the staphylococcus has produced visible

growth before an inhibitory concentration of dye has reached it ; within this zone, inhibition started before growth was sufficiently advanced to be visible. There is plainly a critical time after inoculation dividing these two conditions. When the concentration gradient at the edge of the inhibited zone is steep (*i.e.* m_0 is many times the value of m', which is now the minimum inhibitory concentration) the edge will be sharp, but if the concentration gradient is more gentle (when m_0 approaches m') the edge will be gradual, from no growth on the inside, through step-like increases of growth, to full uninhibited growth on the outside.

Using values of m_0 many times greater than the inhibitory concentration, we first investigated the effect of incubating the plate, inoculated with staphylococcus, for varying lengths of time before the dye was added to the cup. With 0·4 per cent. crystal violet added after 3 hours' incubation the zone of inhibition, though somewhat narrower than that produced by immediate addition of the dye, was sharply defined. When added 6 hours after, the edge was diffuse, and when the dye was not added till 9 hours after, some growth was visible up to the edge of the cup. Addition after 12 hours showed no difference from a plate without the addition of antiseptic. The critical time was from 6 to 10 hours, with 8 hours as the most probable value. The size of the zones of inhibition resulting from adding crystal violet immediately after inoculation should therefore be given by :—

$$\log m_0 = \frac{1}{4D \; 2 \cdot 30} \; \frac{x^2}{8} + \log m',$$

where m_0 is the concentration in the cup, and m' the concentration which only just inhibits growth. If x is measured in millimetres, the value of D must be expressed in mm.²/hr. at 37° C. If d is the diameter of the cup, the diameter of the zone of inhibition $= 2x + d$. Fig. 3 shows the calculated curve with $D = 0 \cdot 30$ mm.²/hr.—the result for surface-inoculated plates. A repetition with poured inoculated plates gave a value of $D = 0 \cdot 23$ mm.²/hr. This value for D should be compared with the value obtained by correcting the formerly determined figure at room temperature to 37° by formula (2), assuming that $\eta_{15°}/\eta_{37°}$ for water can be used for the change in viscosity with temperature for the solute. This correction gives $D = 0 \cdot 19$ mm.²/hr. The experimentally determined values of x for a wide range of values of m_0 are shown and agree very well with the curve. The value of D depends on the composition of the batch of medium used, especially with regard to the percentage of agar and its pre-treatment. The value of m' used is somewhat greater than the inhibitory concentration of crystal violet found in liquid media. This is not unexpected, if it is realised that the antiseptic must be absorbed from the agar into the growing colonies and is not brought to the organism by convection currents as it is in a liquid medium. The inhibitory concentration

varies to some extent with the size of the inoculum. Moreover the curves are calculated on the assumption that the concentration in the
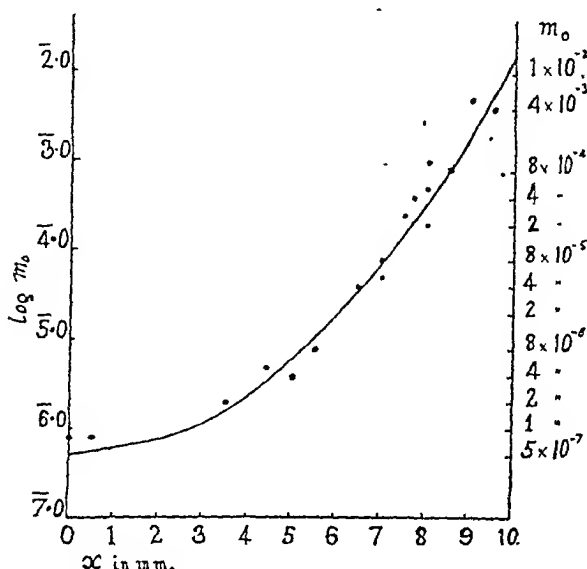
FIG. 3.—Crystal violet inhibition of *Staph. aureus*.

$$D = 0.30 \text{ mm.}^2/\text{hr.}$$
$$t = 8$$
$$\log m_0 = 0.045\, x^2 + \overline{7}.70$$

cup remains constant for some hours (up to 8). With low concentrations in the cup, the antiseptic will be more rapidly exhausted and the effective value of m_0 and m' reduced accordingly.

Penicillin

Having found the applicability of formula (1) to the results of the assay method for crystal violet, the possibility of applying it to explain the published results for penicillin was tested. The value to be assigned to the three symbols t, m' and D must be determined for the conditions of the assay.

The value of t, as we have seen in the crystal violet experiments, is determined by the maximum amount of incubation of the staphylococcus which can be permitted if inhibition of growth is still capable of being detected. As this is determined previously by the rate of growth of the staphylococcus on the untreated plates, we expected the value to be the same as previously found, *i.e.* 8 hours. That this supposition was approximately correct is indicated by the results of Schmidt and Moyer, who give a series of curves for the zone diameters after incubating inoculated plates (for drying purposes) before adding the penicillin. The curve for 7 hours has zones only 3-4 mm. wide around the cup, and it is evident from these graphs

that a period of 8–9 hours would lead to their complete absence. Any factor affecting the rate of growth of the staphylococcus or the length of the lag period before growth proceeds will of course affect this value for t.

The value of m' is given by the intersection of the standard penicillin curve with the concentration axis. Analogous to our results with crystal violet, its value of approximately 0·2 unit is a few times greater than the concentration necessary to inhibit the staphylococcus in liquid media. Both Schmidt and Moyer and Abraham *et al.* give a value of 0·045 unit for the latter.
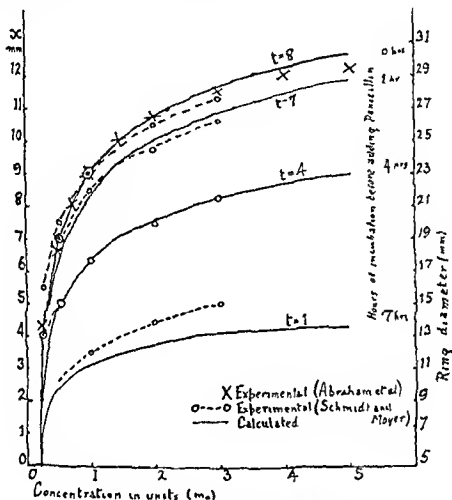
Fig. 4.—Penicillin cup assay.

$$D = 1 \cdot 57 \text{ mm.}^2/\text{hr.}$$

$$\log m_0 = \frac{1}{4D\ 2 \cdot 3} \cdot \frac{x^2}{t} + \log 0 \cdot 20$$

The value of D cannot be directly determined as in the case of crystal violet, but if the values of t and m' are inserted in formula (1) and the position of any single point on the curve known, D may be calculated from the formula. The paper of Abraham *et al.* gives the internal diameter of the cup used as 5·1 mm. and a graph of a standard curve with a ring of 23 mm. diameter for 1 unit ($= m_0$) of penicillin in the cup. It follows that $x = \dfrac{23 - 5 \cdot 1}{2} = 9$

and

$$\log 1 \cdot 0 - \log 0 \cdot 2 = \frac{1}{4D\ 2 \cdot 30}\ \frac{9^2}{8}$$

or

$$D = 1 \cdot 6 \text{ mm.}^2/\text{hr.} = 4 \cdot 4 \times 10^{-6} \text{ cm.}^2/\text{secs.}$$

With these values known it is now possible to calculate a curve
for the relationship between the concentration in the cup (m_0) and
the value of x. The diameter of the ring is then given by $2x+5$. The
calculated curve fits the published experimental curve of Abraham
et al. for all the values of m_0 given ($\frac{1}{4}$–5), within the limits of experi-
mental error (fig. 4). Moreover, using the same formula but with
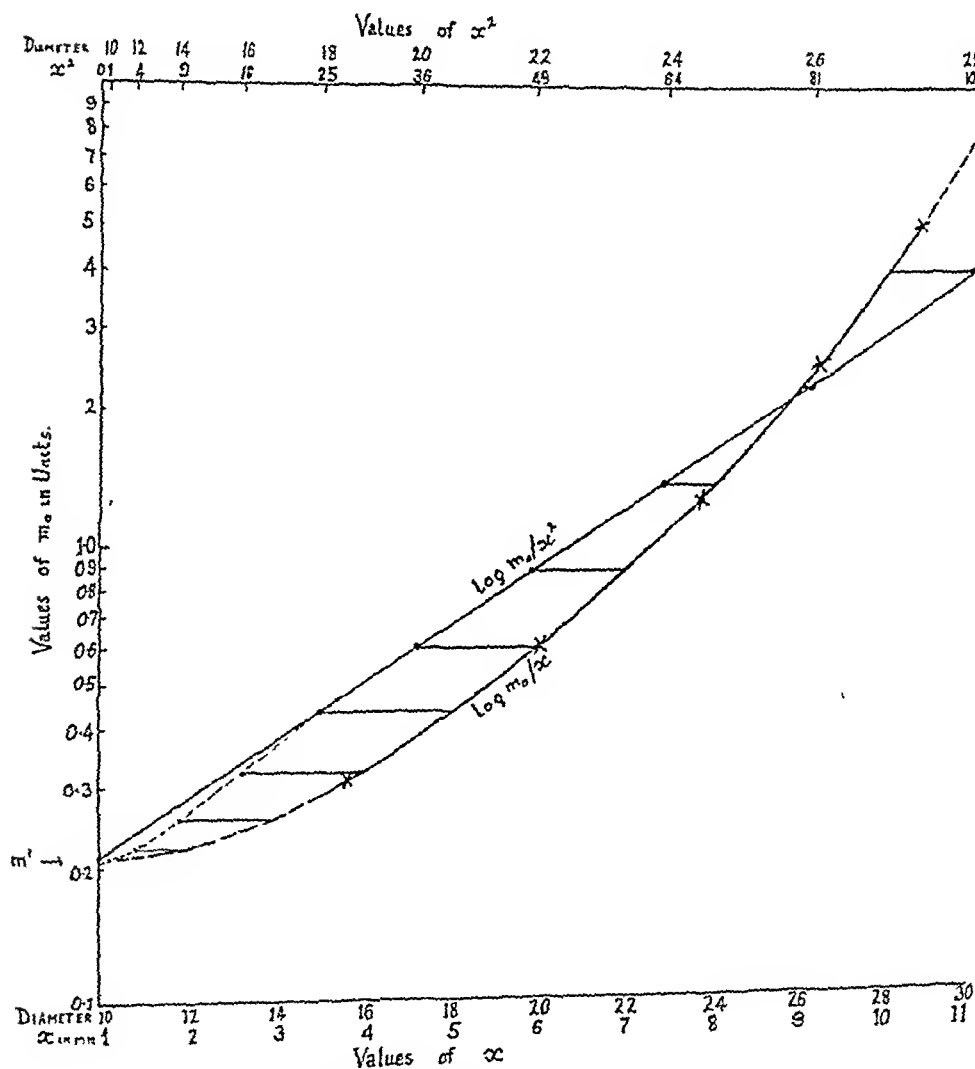
Fig. 5.—Penicillin standard curve in $2\frac{1}{2}$ per cent. nutrient agar.

$$t = 8$$
$$D = 1\cdot06 \text{ mm.}^2/\text{hr.}$$
$$m' = 0\cdot21$$
$$\log m_0 = 0\cdot0128 \ x^2 + \log 0\cdot21$$

values of $t = 6$, 4 and 1, curves may be constructed corresponding to
plates prepared by incubating for 8-6, 8-4 and 8-1 hours before adding
penicillin. These curves are as nearly as possible identical with the

series given by Schmidt and Moyer. They cannot be completely equated with them, because these authors do not state the internal diameter of the cylinder used, which determines x; nor is the diffusion coefficient of their standard curve quite identical with that of Abraham *et al.* The latter difference may be due to a different concentration of agar, the strength of which is not stated in Abraham's paper.

Dr A. H. Campbell, Botany Department, Bristol University, has kindly allowed us to test the applicability of the formula to a number of the daily standard charts prepared to assay unknown penicillins. The product Dt and the value of log m' will vary with the precise conditions employed for each day's assays, but if log m_0 is plotted against x^2 a straight line should result. It is the practice normally to plot m_0 on logarithmic paper against the diameter of the zone of inhibition. One of Dr Campbell's charts is reproduced (fig. 5), and as the diameter of the cup used was 8 mm. the values of x can also be shown. The method of converting the standard curve to give the values for x^2 is illustrated. Agreement with the straight line required by theory is accurate for zones from 16 to 28 mm. and this result is typical of the others we have examined. With concentrations of penicillin below 0·4 unit, the estimated concentration in the cup is slightly inaccurate, but the edge of such zones is more diffuse and difficult to measure accurately. Osmotic changes may operate more notably with small zones also.

The remarkably good agreement obtained means that the standard curve could be constructed with the aid of results for two values of m_0 only instead of the five or six points previously necessary. The values of Dt and m' can also be determined and thus the diffusibility and the antiseptic action separated. This should be of value in comparing the action of different penicillins and in analysing the effects of numerous factors affecting the curve.

The values obtained for the diffusion coefficients in agar would lead us to conclude that the molecule of penicillin must have a smaller radius than that of crystal violet. The compactness of the molecule with its high diffusion rate may be of some importance for facilitating the penetration of animal tissues.

Conclusions

1. The application of a theoretical formula to agar cup assay methods is suggested.

2. This formula has been shown to apply to the diffusion of crystal violet through nutrient agar and can be adapted to explain the inhibition zones on staphylococcus agar cup plates.

3. The formula explains many of the published facts concerning the factors affecting the estimation of penicillin by this method.

4. Its application to penicillin standard curves enables the limits

of their accuracy to be seen and allows a more detailed analysis of the factors affecting (*a*) diffusion and (*b*) antiseptic action.

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SHORT ARTICLES

PLATYMERIA

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(PLATE X)

Platymeria—a flattening of the upper end of the shaft of the femur in an, antero-posterior direction—has been ascribed to various causes such as muscle pull, posture and gait, and activities involved in mountain climbing. Cameron (1934) maintained that platymeria cannot be due to squatting. He suggested that it was due to unwonted strain during childhood and early adolescence. Platycnemia (a flattening of the tibia), he stated, is not necessarily associated with platymeria, but is due to squatting and is related to the expanded origin of the tibialis posterior muscle. Platymeria and platycnemia appear to occur more frequently in primitive modern man and early man in Europe than in more advanced peoples. Dudley Buxton (1038-39) suggested that the femoral column tends to be flattened when there is a shortage of bone material and rounded when there is an abundant supply. While the cause of bone deficiency is difficult to determine, he believed that it might be due to calcium or vitamin deficiencies resulting from the diet peculiar to certain primitive races.

Parsons (1013-14), Holtby (1917-18) and Pearson and Bell (1019) claimed that platymeria was more common in females than in males, and more common in the left than in the right femur. Pearson and Bell (p. 133) write: "The principal part of torsion occurs above the pilastric section and we suggest that this torsion strengthens the bone in the principal plane of bending even as the pilaster does. We look on the reduced torsion of the right femur as to some extent compensated for by increased antero-posterior diameters in the proximal part of the bone".

The amount of platymeria in a bone is expressed as the platymeric index (Manouvrier), namely $\dfrac{100 \times \text{A.P.D.}}{\text{T.D.}}$, where the platymeric antero-posterior diameter and transverse diameter are determined in the infra-trochanteric region. The A.P. diameter is taken first, with the calipers, on the inner side of the gluteal ridge, and so avoiding it. The transverse diameter is next determined, at the same level.

The antero-posterior diameter is usually less than the transverse diameter, so that the average index in normal bones is 85. Occasionally cases are seen (usually associated with much bowing and pilastric development) where the antero-posterior diameter is greater than the transverse and Manouvrier called this transverse platymery. Lehmann-Nitsche (1894-95) proposed the name stenomery for this condition and graded the indices for femora into three main groups :—(1) platymeric—index under 80, (2) eurymeric—index 80-100 (normal), (3) stenomeric—index over 100.

Pearson and Bell (p. 372), referring to Hirsch, state " that the collar angle is sensibly related to the shape of the shaft at the platymeric section—the rounder the shaft, or less platymery, the greater the collar angle. This probably

only means that a low collar (*i.e.* cervical) angle in man, involving the neck springing more horizontally from the shaft, increases the subtrochanteric transverse diameter and so reduces the platymeric index,—that is, increases platymery ".

An examination of bone structure in normal and pathological femora suggests that the pressure of body weight acting on the angulated upper part of the femoral tube (junction of neck and shaft) is important in determining, on mechanical grounds, this relative flattening of the proximal part of the shaft. The normal antero-posterior flattening of the neck is a mechanical adaptation, involving the economic use of material with sufficient strength to support body weight acting on the inclined neck. If the axes of the neck and shaft were a continuous vertical line, a circular cross section of the neck—as of the shaft— would suffice to support the thrust of body weight acting vertically along this single linear axis ; but in the case of a much angulated tube, a considerable filling-in of or support for the concavity of the angle would be required to withstand the bending-moment of body weight—thereby producing an oval section, *i.e.* antero-posterior flattening. It follows, then, that the size of the cervical angle has some effect in deciding not only flattening of the neck but also flattening of the upper part of the shaft.

Depression of the axis of the neck of the femur (decreased cervical angle) in pathological conditions appears to be associated with a low platymeric index, *i.e.* with definite platymeria of the upper part of the shaft. Depression of the neck axis of this nature is evident in cases of osteo-arthritis and osteo-periostitis.

Osteo-arthritis

Fig. 1 shows an osteo-arthritic femur in which the upper surface of the head has been eroded and flattened, while new bone has been laid down on the
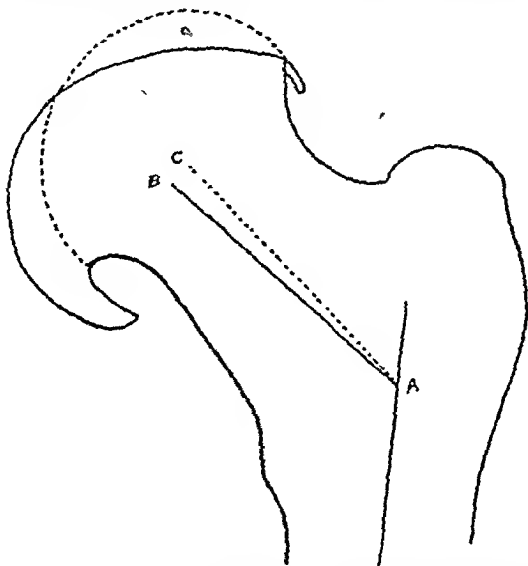
Fig. 1.—Tracing of an antero-posterior radiograph of the upper end of an osteo-arthritic femur. The dotted line represents the original outline of the head, the continuous line the new articulating surface of neck. AC = original axis, AB = new axis. Platymeria is present.

under surface in an attempt to restore its spherical character. This additional bone consists of a superficial compact articular layer and its subjacent supporting

PLATE X

PLATYMERIA

Fig. 2.—Antero-posterior radiograph of pathological femur (osteo-periostitis) showing decreased angle of neck and thickening of the medial and lateral aspects of the upper end of the shaft. Definite platymeria is present.

trabeculæ, which have been laid down on geodetic principles ; these new trabeculæ separate the new superficial compact layer from the original articular surface, which however can still be identified in section or by X-rays (Townsley, 1944).

The axis of the neck (AC in fig. 1), as determined by the position of the original head outline, bisects the base line of the head, though not at right angles. The existing axis (AB), as determined by the new head outline, is nearer the horizontal. The shape of the neck has changed to overcome the mechanical disadvantage of this more horizontal neck axis, the index of ellipticity having fallen from the normal 75 to 68 as a result of the alteration in the ratio of antero-posterior to vertical diameter, i.e. the neck is more oval on section than in the normal bone.

Measurement of the platymeric antero-posterior and transverse diameters also gives an index well below normal—71 as compared with a normal average of 85. Thus there is a platymeric condition of the upper part of the shaft in association with the depression of the articular surface of the head of the femur.

A second specimen of osteo-arthritis showed similar features. The antero-posterior and transverse diameters were determined with calipers in the platy-meric plane, and the platymeric index was found to be 64·8.

Osteo-periostitis

This specimen showed a reduced cervical angle (108°) and a marked alteration in the ratio of antero-posterior to transverse diameter in the subtrochanteric region of the shaft. In this region there is marked protrusion of the medial and lateral walls due to new bone formation, and the surface of the bone is rough (fig. 2), suggesting that periostitis had been present. The platymeric index is 68—that is, there is definite platymeria.

Coronal section and antero-posterior radiography show marked thickening of the compact tissue in the medial and lateral aspects of the upper end of the shaft and in the lower border of the neck. This represents a response on the part of the bone, based on mechanical principles, to oppose the forces of body weight which are more likely to cause collapse or shearing of the neck in its depressed (more horizontal) position. The trabeculæ, which extend from the medial part of the neck and shaft upwards and laterally to the great trochanter and to the lateral part of the neck, are exaggerated, and act as internal pressure-resisting structures which prevent collapse of the tube at its angle.

Beneath the thickened compact layer of the upper lateral part of the shaft there is a layer of bone representing a continuation of the original line of the shaft before the onset of the softening process which resulted in the partial collapse of the neck. Beneath the compact layer on the medial aspect of the upper end of the shaft are several obliquely placed groups of trabeculæ which prolong the line of the lower border of the neck and therefore extend obliquely downwards and laterally into the cavity of the shaft (fig. 2). The radiograph suggests that, as a result of pathological processes, the neck of the femur had collapsed (or, under body weight, had rotated downwards about an antero-posterior axis passing through the junction of the neck and shaft).

In platymeria in general the compact tissue of the medial and lateral walls of the upper third of the shaft is much thicker than that of the anterior and posterior walls. This, however, is merely an exaggeration of the normal (eurymeric) form. The thickening is mechanically determined and provides maximum resistance in the plane of greatest stress. Further, it follows that, at a higher level (i.e. at the level where the compact bone of the medial and lateral walls begins to undergo trabeculation, as seen in a coronal section or antero-posterior radiograph) the number of trabeculæ arising from these thickened

medial and lateral walls will be greater than normal. These extra trabeculae are required to strengthen the neck following depression of its axis, in the same way that the thickening of the medial and lateral compact bone lower down resists the new forces.

Summary

In pathological conditions of the femur which result in depression of the axis of the neck platymeria is found. The platymeric condition of the upper end of the shaft is associated with a decrease in the index of ellipticity of the neck and is a mechanical adaptation to support body weight on the abnormally inclined (more horizontal) neck.

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STIMULATION OF THE GROWTH OF MYCOBACTERIA BY EGG YOLK

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The relative values of white and yolk of egg as media for the cultivation of tubercle bacilli were investigated by Griffith and Griffith (1907), who stated that yolk and whole egg were about equal in value and that both were far superior to the white alone. Corper and Cohn (1933) found that inspissated yolk was superior to whole egg. Other workers have experimented with media containing, in most cases, a higher proportion of yolk than the usual egg mixtures, with or without addition of other nutrient materials (Herrold, 1931; Schwabacher, 1936-37; Corper, 1937-38; Wallenstein, 1941; McCarter and Kanne, 1942; Sasano and Medlar, 1943). Boissevain and Schultz (1938) showed that the growth-promoting factor of yolk is fat-soluble.

The work reported in this paper began with the observation that the growth of tubercle bacilli on an egg-white medium is often so poor that it amounts to no more than a slight thickening of the inoculum. This fact makes it possible to study growth stimulation by adding known amounts of test substances to egg white, which is subsequently coagulated. The growth of pathogenic and saprophytic mycobacteria has been investigated by this means.

METHODS

Preparation of media

Egg fluids—whole egg (for Dorset's medium), yolk and white—were collected separately under aseptic conditions Each was diluted with one third volume of 0 9 per cent NaCl solution, homogenised and filtered through sterile muslin When desired, glycerol was added to make a final concentration of 5 per cent Media were inspissated at 75 80° C for 30 minutes on two successive days, or alternatively heated at 80° C near the top of the steamer for 15 minutes on two successive days Test substances and extracts were added to the diluted egg white before coagulation Media were distributed either in sterile screw capped 1 oz bottles or in test tubes plugged with cotton wool and sealed with paraffin wax after inoculation

" Defatted " egg yolk medium was prepared in a similar manner from yolk fraction A (see below) diluted with one third volume of water, 5 per cent of glycerol being added

Preparation of yolk fractions

Fraction A (water soluble yolk constituents) Yolk diluted with an equal volume of 10 per cent NaCl solution was extracted with successive quantities of cold ether until it was free from lutein (12 extractions) Ether was removed under reduced pressure

Fraction B (ether soluble yolk constituents) Yolk dried with anhydrous Na_2SO_4 was extracted continuously with ether (Soxhlet) until the reflux was colourless The ether was distilled off and residual traces removed from the egg oil *in vacuo*

Fraction C (crude phosphatides) Yolk dried with anhydrous Na_2SO_4 was extracted with hot acetone (Soxhlet) until free from pigment Continuous extraction with hot absolute alcohol followed for 3 hours The alcohol soluble residue (fraction C) was obtained by removal of alcohol under reduced pressure

RESULTS

Comparison of growth on various coagulated egg media

The results of seeding acid fast organisms on various egg media are shown in table I Glycerol was omitted from the media used for cultivation of *Myco tuberculosis bovis* but included in all other cases The inoculum was two loopfuls of a uniform suspension containing 2 4 mg wet weight of bacteria per ml

The amount of growth on Dorset's medium was utilised as a normal standard It will be seen that saprophytic acid fast bacteria and those mycobacteria which are pathogenic for cold blooded animals (except *Myco marinum* Aronson and *Myco tuberculosis*, Cayman strain) grew moderately well on egg white but did not attain the standard of growth characteristic of Dorset's medium *Myco phlei* and *Myco ranœ* also grew moderately well on the partially defatted yolk (fraction A) The avian, bovine and human varieties of the tubercle bacillus exhibited no growth on egg white and defatted yolk media at a time when luxuriant growth had been attained on Dorset's medium All the organisms grew faster on the yolk medium

Myco tuberculosis hominis (strains H 37 and T_1) produced only a scanty growth on egg white medium after ten weeks On serial subculture this scanty growth, in the case of H 37, appeared after the same time, but on transference to Dorset's medium there was immediate stimulation of growth and attainment of the usual standard Strain T_1 failed to grow in subcultures on egg white medium

TABLE I

Growth of mycobacteria on various egg media

Organism	Incubation		Medium			
	Temp. °C.	Time (days)	Dorset	Egg white	Yolk	Defatted yolk
Myco. phlei, no. 525	38	4	+ + + +	+ +	+ + + + + +	+ +
Myco. smegmatis, no. 523	38	4	+ + + +	+ + +	+ + + + + +	...
Myco. stercoris, no. 3820	38	4	+ + + +	+ +	+ + + + + +	...
Myco. karlinski, no. 2071	38	4	+ + + +	+ +	+ + + + + +	...
Myco. sp. leprous, Kedrowsky, no. 509	38	6	+ + + +	+ +	+ + + + + +	...
Myco. butyricum, no. 337	38	5	+ + + +	+ +	+ + + + + +	...
Myco. ranae, no. 2891	38	12	+ + + +	+ +	+ + + + + +	+ +
Myco. marinum Aronson, no. 2275	25 {	7	+ + + +	—	+ + + + +	...
		24	+ + + +	+	...	...
Myco. thamnopheos Aronson, no. 2927	25	5	+ + + +	+ +	+ + + + + +	...
Myco. tuberculosis, Cayman strain, no. 2014	25 {	6	+ + + +	—	+ + + +	...
		16	+ + + +	—	+ + + + +	...
		30	+ + + +	±	...	...
Myco. tuberculosis Chelonei Friedmann, no. 946	25 {	6	+ + + +	—	+ + + + + +	...
		21	+ + + +	+ +	...	...
Myco. tuberculosis hominis (avirulent), T₁ (C.S.L.)	38 {	10	+ + + +	—	+ + + + +	...
		70	...	+	...	...
Myco. tuberculosis hominis, H 37 (C.S.L.)	38 {	10	+ + + +	—	+ + + + +	—
		70	...	+	...	...
Myco. tuberculosis hominis, G. 12 (local strain, recently isolated)	38 {	12	+ + + +	—	+ + + + + +	...
		45	...	—	...	...
Myco. tuberculosis bovis, Rb 15934 (C.S.L.)	38	21	+ + + +	—	+ + + + +	...
Myco. tuberculosis bovis, no. 46	38	17	+ + + +	—	+ + + + +	...
Myco. tuberculosis bovis, St. (local strain)	38	21	+ + + +	—	+ + + + +	...
Myco. avium, T₂ (C.S.L.)	38 {	14	+ + + +	—	+ + + + + +	—
		45	...	—	...	—

Strains designated by a catalogue number without an accompanying alphabetical letter were obtained from the National Collection of Type Cultures, Lister Institute, London.

C.S.L. = Commonwealth Serum Laboratories, Melbourne.

The degree of growth is indicated by the number of plus signs.

Effects of adding nutrients to egg-white medium

The addition of purified ovo-lecithin (Schering-Kahlbaum) and of ether-extractable yolk lipids (fraction B) to egg-white medium stimulated the growth of the organisms listed in table II. Three g. of lecithin were emulsified in 100 ml. of egg white and 3 parts of yolk fat in 4 of egg white. A crude preparation of yolk phosphatides (fraction C) produced the same effect as the commercial lecithin. Addition of small amounts of yolk to egg-white medium stimulated *Myco. tuberculosis hominis*. After incubation for 20 days strain T₁ gave the following results : on Dorset's egg medium (23 per cent. yolk), + + + + + ; on egg white with 10 per cent., 1 per cent. and 0·1 per cent. yolk added, + + +, + + and ± respectively ; on egg white without yolk, no growth.

Addition of the following materials (neutralised where necessary) to egg white failed to stimulate the growth of tubercle bacilli. The strains used are indicated in brackets :—Sodium citrate, 0·37 per cent. (T₁), 0·2 per cent.

(H 37, T₁) ; asparagine, 0·1 per cent. (T₁), 0·05 per cent. (H 37, T₁) ; Ca⁺⁺, 30 μg. per 5 ml. of medium (T₁) ; Fe⁺⁺⁺, 25 μg. per 5 ml. of medium (T₁) ; *dl*-alanine, 0·24 per cent. (T₁) ; creatine, 0·1 per cent. (T₁) ; phthiocol, 25 μg. per 5 ml. of medium (T₁) ; choline, 0·06-0·32 per cent. (H 37, T₁) ; ethanolamine, 0·48 per cont. (H 37, T₁), 0·11 per cent. (H 37) ; Na palmitate, 0·04 per cent. (H 37, T₁) ; Na stearate, 0·24 per cent. (H 37, T₁) ; Na oleato, 0·1-0·37 per cent.

TABLE II

Effects of adding lecithin and yolk fat to egg-white medium

Organism	Incubation		Medium			
	Temp. °C.	Time (days)	Dorset	Egg white	Egg white + lecithin	Egg white + yolk fat
Myco. phlei, no. 525 . .	38	7	+ + + +	+ +	+ + + + +	+ + +
Myco. smegmatis, no. 523 .	38	7	+ + + +	+ + +	+ + + + +	...
Myco. stercoris, no. 3520 .	38	7	+ + + +	+ +	+ + + +	...
Myco. karlinski, no. 2071 .	38	5	+ + + +	+ +	+ + + +	...
Myco. ranae, no. 2891 .	38	12	+ + + +	+ +	+ + + +	...
Myco. marinum Aronson, no. 2275	25	12	+ + + +	—	+ +	...
Myco. tuberculosis, Cayman strain, no. 2014 . .	25	12	+ + + +	—	+ + +	...
Myco. tuberculosis hominis, T₁ (C.S.L.) .	38	12	+ + + +	—	+ +	+
Myco. tuberculosis hominis, M. (local strain) } 38 {	38	12	+ + + +	...	—	...
	38	30	+ + + +	...	+	+ +
Myco. avium, T₂ (C.S.L.) .	38	14	+ + + +	...	+ +	...

(H 37, T₁), 0·5 per cent. (H 37) ; sodium α-glycerophosphate, 0·25 per cent. (H 37, T₁) ; sodium β-glycerophosphate, 0·12 per cent. (H 37, T₁), 0·2 per cent. (H 37) ; ascorbic acid, nicotinamide, pyridoxin, pantothenic acid, and ribo-flavine, each 0·75 mg. per 5 ml. of medium (T₁) ; *dl*-lactate, 0·2 per cent. (T₁) ; cytochrome c, 2·5 mg. per 5 ml. of medium (T₁) ; yolk fraction A, 13 per cent. by volume (H 37, T₁) ; muscle Kochsaft, 20 per cent. by volume (H 37, T₁).

A simple yolk-enriched egg medium

The stimulating effect of yolk is so marked that this material appears to be a suitable basis for the preparation of solid media designed specially for cultivation of tubercle bacilli. Corper (1938) and Wallenstein (1941) have described such media. The proportion of yolk in these and some other published media are approximately as follows.

	Volume of yolk in 100 volumes of medium before coagulation
Wallenstein (1941)	72
Corper (1938)	71
Sasano and Medlar (1943)	43
Dorset (usual whole egg prescription) . .	23
Loewenstein-Jensen (Jensen, 1932) . .	20
Potragnani (1920)	17
Herrold (1931)	15

A diluted yolk medium containing about 70 volumes of yolk per cent. has one serious disadvantage : it dries and cracks during inspissation and, although it retains its moisture when sterilised by steaming, it loses water unduly on

storage, even at low temperatures, and during incubation. This difficulty can be overcome by including a certain amount of egg white with the yolk.

Experiments were conducted with egg media containing 20, 40, 50, 60, 65 and 72 por cent. of yolk by volume. A small inoculum (1 drop of a suspension containing 10^{-5} mg. bacteria per ml.) of *Myco. tuberculosis hominis* (H 37) was used in each case. The efficiency of the medium increased with the percentage of yolk, until a maximum was attained when the yolk content was 60-65 volumes per cent. The lag period was not diminished by raising the yolk proportion to 72 volumes per cent., and the addition of milk, potato, asparagine and citrate, singly and in combination, did not enhance growth significantly or shorten the lag period.

On these grounds a medium of the following composition was made and tested with clinical material :—

							Volumes
Egg yolk	.	.	.	.	.	.	60
Egg white	.	.	.	.	.	.	15
0·9 per cent. NaCl solution	.	.	.	.	.	.	22
Glycerol (pure)	.	.	.	.	.	.	1
1 per cent. Congo red solution	.	.	.	.	.	.	1
1 per cent. malachite green solution	.	.	.	.	.	.	1
							100

On this medium growth of *Myco. tuberculosis hominis* appeared within 14 days and attained full proportions in 3-4 weeks ; *Myco. tuberculosis bovis* appeared within 3-4 weeks and attained full growth in 5-6 weeks. Congo red is included to provide a background against which early colonies are visible. The inclusion of 1 volume of 1 per cent. malachite green solution reduces the number of contaminated tubes to normal proportions. For culturing bacilli from heavily contaminated specimens such as gastric, contents, sputum and post-mortem material, the amount of malachite green is increased to 3 volumes and the Congo red omitted.

No advantage was gained in cultivating *Myco. tuberculosis hominis* by increasing the concentration of glycerol above 1 volume per cent. The yolk-enriched egg medium retains moisture satisfactorily when sterilised near the top of the steamer at 80° C. for 15 minutes on two successive days.

The yolk-enriched egg medium has been used in this laboratory for the primary cultivation of tubercle bacilli from all kinds of clinical and post-mortem material. It has proved at least equal in value to the more complicated media of Loewenstein-Jensen and Petragnani with respect to time of appearance of colonies, luxuriance of growth, detection of small seedings and differentiation of type. The medium is favourable to the growth of *Myco. tuberculosis bovis.*

DISCUSSION

The fact that acid-fast saprophytes grow moderately well on inspissated egg white, although tubercle bacilli do not, is evidence of their simpler nutritional requirements. Edson and Hunter (1943) found a similar difference when the same organisms were cultured in a fluid synthetic medium. Furthermore, the stimulating effect produced by the addition of yolk and yolk extracts to egg white is relatively greater in the case of tubercle bacilli. A stimulating factor is associated with the phosphatide fraction of the yolk. A crude *phosphatide* fraction and a commercial sample of purified lecithin were about equal in potency. Certain phosphatide constituents, choline, ethanolamine and glycero-phosphates, added to egg white singly and in combination, were ineffective. A complete chemical separation of egg-yolk lipids is necessary for elucidation of the problems raised.

SUMMARY

1. The growth of tubercle bacilli (avian, human and bovine) on an egg white medium is extremely poor, whereas acid fast saprophytes grow more profusely. The addition of sufficient egg yolk stimulates growth of the pathogenic species.

2. A growth stimulant is present in a crude phosphatide fraction of the yolk ; purified commercial lecithin is also active.

3. A simple and efficient yolk enriched egg medium suitable for routine laboratory use is described.

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578 . 65

THE DEMONSTRATION OF CERTAIN FATTY SUBSTANCES IN PARAFFIN SECTIONS

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Several standardised techniques are available for the demonstration of fats and fatty substances in tissues ; these have been most thoroughly reviewed by Lison (1936). With the exception of certain of Ciaccio's methods (quoted by Lison) and the use of osmium tetroxide paraffin sections cannot be used It is the purpose of this note to describe a method of fixation which allows subsequent dehydration of tissue by acetone or the alcohol xylol method and imbedding in paraffin ; the sections cut are stained by sudan black. With this method certain at least of the lipins are preserved and demonstrated Other sections similarly handled can be stained with hematoxylin and eosin, van Gieson, trichromo or one of the stains for elastic tissue such as acid orcein or Verhoeff's hæmatoxylin.

storage, even at low temperatures, and during incubation. This difficulty can be overcome by including a certain amount of egg white with the yolk.

Experiments were conducted with egg media containing 20, 40, 50, 60, 65 and 72 per cent. of yolk by volume. A small inoculum (1 drop of a suspension containing 10^{-5} mg. bacteria per ml.) of *Myco. tuberculosis hominis* (H 37) was used in each case. The efficiency of the medium increased with the percentage of yolk, until a maximum was attained when the yolk content was 60-65 volumes per cent. The lag period was not diminished by raising the yolk proportion to 72 volumes per cent., and the addition of milk, potato, asparagine and citrate, singly and in combination, did not enhance growth significantly or shorten the lag period.

On these grounds a medium of the following composition was made and tested with clinical material :—

	Volumes
Egg yolk	60
Egg white	15
0·9 per cent. NaCl solution	22
Glycerol (pure)	1
1 per cent. Congo red solution	1
1 per cent. malachite green solution	1
	100

On this medium growth of *Myco. tuberculosis hominis* appeared within 14 days and attained full proportions in 3-4 weeks ; *Myco. tuberculosis bovis* appeared within 3-4 weeks and attained full growth in 5-6 weeks. Congo red is included to provide a background against which early colonies are visible. The inclusion of 1 volume of 1 per cent. malachite green solution reduces the number of contaminated tubes to normal proportions. For culturing bacilli from heavily contaminated specimens such as gastric contents, sputum and post-mortem material, the amount of malachite green is increased to 3 volumes and the Congo red omitted.

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DISCUSSION

The fact that acid-fast saprophytes grow moderately well on inspissated egg white, although tubercle bacilli do not, is evidence of their simpler nutritional requirements. Edson and Hunter (1943) found a similar difference when the same organisms were cultured in a fluid synthetic medium. Furthermore, the stimulating effect produced by the addition of yolk and yolk extracts to egg white is relatively greater in the case of tubercle bacilli. A stimulating factor is associated with the phosphatide fraction of the yolk. A crude phosphatide fraction and a commercial sample of purified lecithin were about equal in potency. Certain phosphatide constituents, choline, ethanolamine and glycerophosphates, added to egg white singly and in combination, were ineffective. A complete chemical separation of egg-yolk lipids is necessary for elucidation of the problems raised.

(i) The technique utilises a lipin precipitant—acetone—for dehydration after a fixative designed to prevent lipins from going into colloidal solution

(ii) The material preserved in the sections does not stain with either sudan IV or osmic acid but does stain with sudan black

(iii) Myelin (containing lipins) was stained by this technique

It may be mentioned that this method seemed to be suitable for the demonstration of mitochondria It is true that the whole lipin content of the cells is stained but in the normal kidney the cellular lipin includes little else besides the mitochondria The Golgi element appears in those cells where the mitochondria are few or deficient and in the macula densa of the rabbit kidney the characteristic reversal of the Golgi element is well seen The adrenal shows an interesting picture, as do various other organs

In the kidney the ease of demonstration of mitochondria makes this the method of choice in studies of renal cytology If some method could be developed to suppress the staining of mitochondria, the Golgi element in the cells would be studied with equal facility The method has the desiderata of ease and adaptability and lacks the sources of error inherent in methods using silver and osmium

Summary

A technique is described using cobalt calcium formol fixation, acetone or alcohol dehydration and paraffin imbedding, sections being stained with sudan black For reasons given it is believed that the lipoid stainable by this method includes certain lipines

The use of the method to stain mitochondria in the kidney is described, as well as myelin in the central nervous system Cytological uses are suggested It is not proposed as a histochemical test for lipines

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A CASE OF MULTIPLE TUBERCULOMATA OF THE LIVER

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From the Department of Pathology, Kent and Canterbury Hospital

(PLATE XI)

Tuberculosis of the liver occurs in three anatomical forms   (1) miliary tuberculosis, (2) massive tuberculomata or cold abscesses, and (3) tubular tuberculosis or tuberculosis of the bile ducts (Rosenkranz and Howard, 1936). Massive tuberculosis of the liver, of which the case here presented is an example, is a rare disease   during the last ten years only one other case has been described in the English literature (Pagel, 1938). In America Morris (1930) described one case and collected eleven others from the literature   Only one case was reported as a result of a questionnaire which Morris sent to a number of sanatoria and hospitals and to leading pathologists, representing 11,455 beds. Rosenkranz

and Howard reported three further cases of tuberculous abscesses of the liver and Herrell and Simpson (1938) a case of solitary tuberculoma associated with recurrent hyperpyrexia.

CASE REPORT

Clinical history

A male, aged 42 yrs., was admitted in Feb. 1943 to Ramsgate Hospital with cough and shivering attacks of two months' duration. No tubercle bacilli were found in the sputum at this time. X-ray examination of the chest suggested primary atypical pneumonia at the left base. W.R. negative. There was intermittent pyrexia, rising in the evening to about 101. The pulse rate ranged between 100 and 130. On 15th March he was transferred to the Kent and Canterbury Hospital under the care of Dr Treble. On examination he was pale and thin, with a slightly icteric tinge and malar flush. Temp. 100°, pulse 122, respiration 28. Expansion of the chest was poor and there was slight impairment of the percussion note, with bronchial breathing and coarse rales at the left base. There was a very large palpable spleen and a greatly enlarged liver in which soft nodular masses about $2\frac{1}{2}$ inches in diameter could readily be palpated. No ascites and no abnormality per rectum. X-ray examination of the chest showed obliteration of the left costo-phrenic angle. Kidney shadows and excretion were normal. The blood showed a moderate microcytic hypochromic anæmia and subsequently a leucopenia of 2800 developed. Hijmans van den Bergh reaction normal. On 14th April he was found to have ascites and paracentesis was performed. The fluid was clear, sterile and greenish yellow : no tubercle bacilli and no malignant cells were found. During this time the sweating, rigors and swinging temperature of 102-103° continued without intermission. Occult blood was found in the stools on three occasions. All other investigations were negative. On 10th May he developed generalised petechiæ, dyspnœa became marked and there were coarse rales and bronchial breathing all over the chest. The radiologist reported miliary tuberculosis in both lung fields. The patient died next day, 21 weeks after the onset of the illness.

Autopsy

The body was that of a pale wasted middle-aged male with generalised petechiæ and abdominal distension. The *pleural cavities* contained a moderate excess of fluid ; there were no adhesions. Both *lungs* were firm, solid and riddled with small miliary tuberculous nodules. There was no evidence of an old or recent primary tuberculous focus. *Heart* : slight excess of pericardial fluid ; left sided hypertrophy. *Abdomen* : marked ascites but no peritonitis. Situated at the porta hepatis, around the cœliac axis and along the splenic vein were several enlarged glands showing tuberculous cavitation. *Liver* greatly enlarged (wt. 88 oz.). It contained about 50 large well circumscribed circular masses about 1-1½ inches in diameter. They were encapsulated by dense fibrous tissue and could not be shelled out. On section they showed a honeycomb appearance (fig.), with pus exuding from the meshes. A few were bile-stained. Many tubercle bacilli were found in a smear of the pus. *Spleen* very greatly enlarged (wt. 56 oz.). It was riddled with small irregular punched out cavities, larger than the lesions in the lung and about ⅓ inch in diameter. *Intestines* contained a large quantity of free blood which had come from a chronic tuberculous ulcer in the jejunum. There were also two ulcers in the colon. There were calcified tuberculous mesenteric glands in the ileo-cæcal angle but no evidence of recent tuberculosis. The right *kidney* contained a small tuberculous abscess. All other organs were healthy. Large numbers of tubercle bacilli were demonstrated in sections from all the organs showing gross tuberculous lesions. Sections of the liver showed also

MULTIPLE TUBERCULOMATA OF LIVER

FIG —Photograph of the liver on vertical section, showing the honeycomb appearance
of the multiple tuberculomata

diffuse cirrhosis and fatty degeneration, with many tuberculous lesions ranging in size from minute miliary foci to large caseating abscesses surrounded with moderately thick fibrous tissue capsules. The periportal connective tissue was especially affected and many tuberculous nodules were seen adjacent to branches of the portal vein ; in some cases granulation tissue extended into the lumen of these vessels. The bile ducts appeared healthy.

DISCUSSION

In the case described no primary tuberculous complex could be demonstrated but a calcified gland was found in the ileo-cæcal region. It is possible that this old focus became active and that bacilli escaped into the portal vein and peri-portal lymphatics and so spread to the liver and spleen, the case terminating as a miliary tuberculosis by extension into the hepatic veins. The presence of caseous glands in the porta hepatis and along the splenic vessels supports this view. A similar source and mode of extension were reported by Randolph (1930) in a case of acute miliary tuberculosis limited to the liver. It is interesting to note that Esser (1926) found in his case a type of organism intermediate between the human and the bovine types. In our case the organism was found by Dr H. Lowonthal to be of human type. It has been stated that tuberculosis of the liver is more common in races not possessing natural immunity to tuberculosis, and Gruber (1920) noted fresh caseation of all lymph glands and bile-stained abscess cavities in the liver in autopsies on Senegal negroes. In the differential diagnosis of the present case both actinomycosis and tuber-culosis of the liver were considered clinically, but since these conditions have no characteristic symptomatology, conclusive diagnosis was impossible. The outstanding symptom was the irregular temperature with severe rigors, as in the cases of Morris, and Herrell and Simpson. The greatly enlarged spleen and liver and the hypochromic anæmia with leucopenia suggested a possible diagnosis of splenic anæmia as in the case of Goldberg (quoted by Morris). In Morris's series of 11 cases only one was diagnosed clinically and that only after a laparotomy (Robinson, 1924).

SUMMARY

A case of multiple tuberculomata (massive tuberculosis) of the liver in a man of 42, terminating with miliary tuberculosis, is described. It is suggested that the probable source of infection was the reactivation of an ancient focus in a mesenteric lymph gland, with subsequent spread by the portal vein and periportal lymphatics to the liver and spleen. The literature on the subject is briefly reviewed and the rarity of the condition emphasised.

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A NEW SPECIES OF SHIGELLA

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A newly recognised species of Shigella is described in the present report. Eight strains of the organism were isolated from specimens of human stools examined during the early part of September 1944 at five different army medical organisations. Six of the strains were isolated in France at a time when the occurrence of diarrhœal diseases was not infrequent and when the culturing of stools was done only when clinical findings indicated the usefulness of such procedure. Another strain was isolated from a soldier in England who was hospitalised for vague abdominal distress without diarrhœa. The last strain came from a patient, also in England, who had mucous colitis with intermittent diarrhœa extending over a period of three months. Amœbiasis was ruled out in this last patient by repeated microscopic examination of the stools. The newly recognised organism will be referred to in this communication as *Shigella etousæ* (nov. spec.).

Cultural characteristics

Growth and morphology. The organism is a facultative aerobe which grows luxuriantly within 24 hours in ordinary media at 37° C. On Bacto-tryptose agar plates the colonies are opaque, smooth and slightly convex and range in size from 1 to 2 mm. Opaque rough colonial variants occur and may be obtained in pure culture by selective transplants. Both smooth and rough cultures produce a homogeneous suspension in broth. The organism is a Gram-negative, non-motile, non-sporing plump bacillus of varying length.

Biochemical. Carbohydrate medium was prepared with beef extract broth at pH 7·6, containing 1 per cent. of the desired sugar and brom-cresol purple as indicator. The media were sterilised by Seitz filtration and dispensed aseptically in small metal screw-capped bottles. The Voges-Proskauer reaction was performed on cultures grown in Bacto-MR-VP medium; 0·6. c.c. of 5 per cent. α-naphthol in absolute ethyl alcohol and 0·2 c.c. of 40 per cent. potassium hydroxide were added to 1 c.c. amounts of culture. Utilisation of citrate by the organism was determined by inoculation of Simmons's citrate agar medium (Technical manual, 1941) which was subsequently incubated for 21 days.

The eight cultures of this organism, designated strains 1-8, were found to possess identical biochemical properties. Acid but no gas was produced in glucose, galactose, mannitol, xylose, sorbitol and arabinose within 24 hours. Acid occurred in maltose within 7-14 days and slight acid in dextrin and glycerol within the same period. When 1 per cent. glucose extract broth (brom-cresol purple indicator) was seeded with a small inoculum from a straight wire and incubated at 45° C. for 48 hours, growth occurred but no acid developed (Eijkman test). No acid was produced in lactose, sucrose, salicin, dulcitol, rhamnose, inulin, starch or inositol during 21 days' incubation. Litmus milk became acid 24 hours after inoculation but no clot was formed, the media returning to a neutral reaction between the 14th and 21st days.

The organism gave a negative Voges-Proskauer reaction but the methyl-red reaction was positive. All strains readily formed indole in Bacto-tryptose broth and did not utilise citrate, as evidenced by the lack of growth on Simmons's

citrate agar. Gelatin was not liquefied, hydrogen sulphide was not formed and urea was not hydrolysed. All strains reduced trimethylamine oxide to trimethylamine (Weil and Black, 1944).

Serological characteristics

Methods. Antisera against strains 1 and 2 were prepared in rabbits. Vaccines for immunisation were made from 18-hour extract-broth cultures which had been inoculated from smooth colonies. Broth cultures were centrifuged and the sediment re-suspended in physiological saline solution containing 0·3 per cent. formaldehyde (U.S.P.). The organisms were washed a second time in the same way, and finally the turbidity was adjusted to a barium sulphate nephelometer standard no. 3 (Diagnostic procedures and reagents, 1941). The animals were injected intravenously at 5-day intervals with formolised suspensions of bacteria given in increasing amounts from 0·5 to 3·0 c.c. Sera with satisfactory titres were obtained after four injections.

Antigens for agglutination tests were prepared from 18-hour tryptose agar slant cultures. The growth was washed off with physiological saline solution containing 0·3 per cent. formaldehyde and the turbidity was adjusted to the nephelometer standard no. 3.

The agglutination tests were set up with the usual serial dilutions (1 : 20 to 1 : 5120) and read after incubating in a 55° C. water-bath overnight.

The following procedure was used to prepare absorbed sera. The mass growth from seven tryptose agar plates (24 hours' incubation) was suspended in about 25 c.c. of 0·3 per cent. formol-saline, centrifuged and re-suspended in 9 c.c. of the diluent. One c.c. of antiserum was added, mixed thoroughly and the mixture incubated at 37° C. overnight. After clarifying by centrifugation, the diluted serum was absorbed a second time by adding to it the washed bacterial sediment from a second batch of seven agar plates and treating as before. Agglutination tests with the absorbed serum were set up in the same manner as with the unabsorbed serum.

Homologous agglutination. Results obtained with unabsorbed *Shigella etousæ* antisera 1 and 2 and the test organisms (*Shigella etousæ*, strains 1-8) indicate close antigenic similarity between all strains. All agglutinated to a titre of 1 : 1280 with both sera.

Results obtained with absorbed serum showed no apparent antigenic differences between the strains. Antiserum 1 absorbed with strains 1 or 2 failed to agglutinate strains 1-8. Similar results were obtained after absorption of antiserum 2.

Heterologous agglutination. Cross agglutination tests were performed in order to determine whether there was any antigenic relationship between the new strains and known Shigella species. Known cultures and their homologous antisera obtained from several sources were checked for specificity and for potency before being used in the present studies. The following sera were tested with *Shigella etousæ*, strains 1-8, and the results showed no agglutination reactions in any dilutions of sera tested (1 : 20 to 1 : 2560) :—*Shigella paradysenteriæ* V, W, X, Y, Z, Boyd 103, Boyd P 119, Boyd 88, Boyd 170, Boyd P 288, Boyd P 274, Boyd D 1, Boyd D 19, Boyd P 143, *Sh. ambigua, madampensis, allalescens, sonnei* (rough), *sonnei* (smooth), *dysenteriæ*, and the Sachs antisera (Q 902, Q 1167, Q 771, Q 1030, Q 454, A 12, B 105, B 81). Antiserum against *Shigella etousæ* no. 1 failed to agglutinate suspensions of the Shigella organisms of the 26 strains used to prepare the antisera mentioned.

The α-antigen of Stamp and Stone (1943-44), which has been found in many Gram-negative bacilli (paracolon bacillus, *Aerobacter aerogenes, Proteus morganii*), was not demonstrated in any of the eight strains of *Shigella etousæ*, nor have we found the α-agglutinin in the two *Shigella etousæ* antisera tested. In testing for these factors we used *Aerobacter aerogenes* 1084 antiserum, which contained

the α-agglutinin in 1 : 250 dilution, and the α-antigen-bearing strain of *Proteus morganii*, both of which were kindly supplied to us by the Emergency Vaccine Laboratory, Royal Army Medical Corps.

When the present studies indicated that a new species of Shigella had been encountered, cultures and antisera were submitted to Lt.-Col. A. E. Francis, Emergency Vaccine Laboratory, Royal Army Medical School. He observed that cross agglutination reactions were obtained with *etousæ* antiserum and a Shigella organism recently isolated by Dr Joan Taylor, Emergency Public Health Laboratory Service, Oxford, who had obtained a number of such strains from a dysentery outbreak in a mental hospital (described in the succeeding paper : Lavington *et al.*, 1946). Dr Taylor has furnished us with one of these strains, which we found to be antigenically similar, by agglutination tests with unabsorbed and absorbed *Shigella etousæ* antiserum, to the strains we have isolated.

Pathogenicity

Shigella etousæ strain 1 was tested for mouse pathogenicity. Approximately 3000 organisms per c.c. in 3 per cent. gastric mucin injected intraperitoneally produced death within 48 hours. The infecting organism was recovered from the blood stream.

In the course of these pathogenicity experiments, one of us (S. G. W.) became ill. After a 2-day incubation period he complained of headache and chills and showed a temperature of 99° F. During the 2nd day of illness the temperature returned to normal and he experienced sharp epigastric pains and diarrhœa which continued into the 3rd day, when ten stools were passed. Microscopic examination at this time showed mucus and numerous pus cells but no red blood cells. The abdominal pains subsided on the 4th day but diarrhœa continued. Stool cultures were positive for *Shigella etousæ* at this time. Six stools were passed on the 5th day, with a few accompanying mild abdominal pains. Stool examination showed mucus, a few red blood cells and numerous white cells. He became relatively symptomless after the 7th day, although traces of mucus were found in the stools up to the 10th day. The stools remained positive for *Shigella etousæ* up to the 10th day but from the 11th day onward were negative. He received no symptomatic or definitive treatment. An agglutination test with serum taken on the 14th day gave a positive result in a dilution of 1 : 20 with *Shigella etousæ* no. 1.

Summary

1. The biochemical and serological properties of eight bacterial strains isolated from human stools are described. These properties indicate that these strains belong to the genus Shigella but that they bear no apparent antigenic relationship to species already described.

2. Presumptive evidence is given to indicate that the newly described organism is pathogenic.

3. The name *Shigella etousæ* (nov. spec.) is proposed for this bacterium.

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AN INSTITUTIONAL OUTBREAK OF DIARRHŒA DUE TO A HITHERTO UNDESCRIBED DYSENTERY BACILLUS

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In common with most mental hospitals in this country during recent years, this hospital has experienced outbreaks of dysentery. Previous outbreaks have been due to *Sh. flexneri*, but sporadic cases due to *Sh. sonnei* have also occurred. On 5th September 1944 a case of clinical dysentery was observed in a ward of patients evacuated from another hospital, and within the ensuing week five more cases were reported, two of which were in the same ward as the first. During the succeeding weeks the outbreak spread and, in spite of every precaution, continued to progress. The incidence of new cases was as follows :—

1st week	6	new cases occurring in 4 wards					
2nd ,,	1 ,,	,,	,,	,,	1	,,
3rd ,,	4 ,,	,,	,,	,,	3	,,
4th ,,	3 ,,	,,	,,	,,	2	,,
5th ,,	16 ,,	,,	,,	,,	4	,,
6th ,,	12 ,,	,,	,,	,,	4	,,
7th ,,	6 ,,	,,	,,	,,	3	,,
8th ,,	25 ,,	,,	,,	,,	8	,,
9th ,,	19 ,,	,,	,,	,,	6	,,
10th ,,	13 ,,	,,	,,	,,	7	,,
11th ,,	9 ,,	,,	,,	,,	5	,,
12th ,,	2 ,,	,,	,,	,,	2	,,
13th ,,	4 ,,	,,	,,	,,	3	,,
14th ,,	1 ,,	,,	,,	,,	1	,,
15th ,,	1 ,,	,,	,,	,,	1	,,

With four exceptions, all the cases were confined to one building, the exceptions being two female patients who worked in the laundry but were resident in the annexe, and the only two male cases, both of whom worked in the foul laundry disinfecting station.

Clinical findings

The onset of the disease was sudden, the patients complaining of diarrhœa, vomiting and malaise ; in a number of cases vomiting was the first symptom. Nearly all suffered from colicky abdominal pain and a few from tenesmus. Temperatures ranged from 99° to 101° F., though a few were as high as 104° F. Some patients looked ill, and were pale, clammy and lethargic. In the majority of cases the temperature became normal in 24-48 hours and diarrhœa ceased in 3 or 4 days. All the cases were treated with sulphaguanidine. There were three fatal cases in elderly persons, but in these the dysentery was not the principal cause of death. At autopsy all three showed an atrophic intestinal mucosa, no ulcers were visible, but there were circumscribed areas of congestion in the pelvic colon and in two cases the pelvic colon and rectum contained increased amounts of mucus.

Laboratory findings

The faeces from 122 clinical cases were examined. All showed the presence of mucus, with macroscopic blood in about 25 per cent. ; occasionally only a few flecks of blood were observed. One common feature which has not been

noted in previous outbreaks of dysentery in this institution was the presence of relatively unaltered bile. The fæces were cultured on MacConkey's agar, non-lactose fermenting colonies being subcultured and their biochemical reactions tested. Non-motile Gram-negative bacilli which failed to ferment lactose and sucrose but fermented glucose and mannitol with the production of acid only were isolated from every case. Strains isolated from 14 cases failed to produce indole and proved to be *Sh. sonnei*. The remaining 108 strains gave the same colonial appearance, produced indole, were not agglutinated by standard polyvalent Flexner sera and did not correspond to any hitherto recorded species. Fifteen of these strains were examined in greater detail in the Salmonella Reference Laboratory.

Blood was taken from 20 of the 108 cases from whose fæces the unidentified organism had been isolated; with two exceptions the sera agglutinated the homologous strain, as well as strains isolated from other patients, to titres ranging from 1 : 40 to 1 : 160. Thirty-two sera sent in for the Wassermann reaction were used as normal controls and failed to agglutinate these organisms in a dilution of 1 : 10 and over.

Characters of the new organism

This new organism was an aerobic, Gram-negative, non-motile, non-sporing, non-capsulated rod 2-4·5 μ long and 0·8-1·0 μ in width. On nutrient agar two types of colony were produced. The first was a semi-opaque, circular, low convex colony with a smooth shiny surface and an entire edge, 0·5-1·5 mm. in diameter at 24 hr. and up to 3·0 mm. at 48 hr. The second type was less opaque—a low convex colony up to 2·0 mm. in diameter at 24 hr. and up to 3·5 mm. at 48 hr., with a radially striated matt surface and a crenated edge. The colony became somewhat irregular in outline after 2 days' growth. On MacConkey's agar and on desoxycholate-citrate agar (Hines's modification of Leifson's medium) the colonies were smaller and the characters less marked than on nutrient agar. Both types of colony, however, could still be distinguished on these media.

The biochemical reactions were tested on a peptone water base containing Andrade's indicator, to which 0·5 per cent. of the test carbohydrate was added. Dextrose, galactose, arabinose, xylose, trehalose, mannitol and sorbitol were fermented with the production of acid but no gas in 1 day. Lactose, sucrose, raffinose, rhamnose, adonitol, dulcitol, inositol, salicin and inulin were not fermented in 21 days. Maltose was usually not fermented, but in a few cases there was acid production without gas after 10 days. Litmus milk, after transient slight acidity lasting 1-2 days, became alkaline after the 10th day. Gelatin was not liquefied. Indole was produced from peptone water. Citrate was not utilised as the sole source of carbon. The Voges-Proskauer reaction was negative and the methyl red test positive. H_2S was produced in small amounts; when tested for in lead acetate agar, blackening along the line of the stab was visible in 7 days and this spread into the medium on further incubation. Occasionally its presence could be detected after 1 day's incubation, though 3-4 days was the more usual time on liver agar with lead acetate papers.

Suspensions of the organism failed to agglutinate with the following antisera (titres 1 : 250-1 : 500), both on the slide and in the test-tube, in dilutions of 1 : 25 and over :—*Sh. flexneri* V, W, X, Y, Z, 103, P 119 and *newcastle* ; *Sh. boyd* 170, P 288, D 1, P 274, P 143 and D 19 ; *Sh. shigæ* ; *Sh. schmitzi* ; *Sh. sonnei* ; *Sh. dispar* and *Sh. alkalescens* (four sera from different strains). A rabbit was therefore inoculated intravenously with graduated doses of a formolised suspension made from one of the strains. The serum so obtained was found to agglutinate suspensions made from both types of colony in a dilution of 1 : 400. Fourteen other strains isolated from patients during the same epidemic were agglutinated to the full titre of the serum. This serum failed to react in dilu-

tions of 1 : 25 and over, with suspensions of *Sh. flexneri* X, Y, Z, 103 A, P 119 A and *newcastle*; *Sh. boyd* 170, D 1, P 274, P 143, D 19 and provisional type (1296/7); *Sh. shigæ*; *Sh. schmitzi* and *Sh. sannei* (smooth): it agglutinated *Sh. flexneri* W, WX and P 119 B and *Sh. boyd* P 288 at 1 : 25 but not at 1 : 50, and *Sh. flexneri* V and 103 B and *Sh. sannei* (rough) at 1 : 50 but not at 1 : 100. These findings were regarded as not significant owing to the presence of natural agglutinins in rabbits' sera (Boyd, 1939-40).

Four strains were used to absorb the serum, and in every case they removed the agglutinins for the homologous strain.

Through the courtesy of Lieut.-Col. A. E. Francis of the Emergency Vaccine Laboratory, eight strains of an undescribed organism suspected of causing clinical dysentery, which had been identified by Major G. Heller of the 1st Medical General Laboratory, U.S. Army, were also examined. Major Heller was kind enough to supply us with some antiserum against one of his strains. These strains, which were isolated from various sources, were biochemically and serologically identical with the fifteen strains isolated from the epidemic described above. Absorption of Major Heller's serum with one of our strains and of our serum with one of his strains removed all the agglutinins from the serum against its homologous organism.

Summary

An outbreak of clinical dysentery in a mental hospital has been described in which all the affected patients showed a similar clinical picture. An organism with the morphological, cultural and biochemical reactions of a dysentery bacillus was isolated from 122 cases, of which 14 were *Sh. sonnei* and 108 proved to be a new species. The macroscopic and microscopic appearances of the fæces were also suggestive of bacillary dysentery. The sera of 20 cases from whose fæces the new organism had been isolated were examined; 18 contained agglutinins to the organism in significant amounts.

Fifteen of the 108 strains were examined in detail and have been shown to belong to a new species identical with that described in the preceding paper (Heller and Wilson, 1946) and named by them *Shigella etousæ*.

From the epidemiological, clinical and bacteriological findings it has been shown that *Sh. etousæ* is a cause of bacillary dysentery in man in this country. Serologically it bears no relationship to any previously described dysentery bacillus and differs from members of the *flexneri* group in its ability to ferment xylose and failure to ferment rhamnose.

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A CASE OF ISLET-CELL TUMOUR OF PANCREAS

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(Plate XII)

The last three decades have seen the discovery of insulin and the beginnings of our present ideas on hyperinsulinism. Harris (1924) described the treatment by carbohydrate alimentation of a small series of cases with symptoms of hypoglycæmia and abnormally low blood sugar levels. In 1927, Wilder *et al.* recorded the first instance of a verified insulin-producing tumour of the pancreas composed of islet tissue. Two years later Howland *et al.* (1929) published the first report of a successful operative removal of an islet-cell tumour. Since then, the number of cases successfully treated by operation has risen to fifty-six (Walker and Boger, 1945) and the total of all reported cases must be over one hundred.

A further case is now reported in which studies of the blood sugar levels were made both before and after operation.

Clinical history

The patient, a married woman æt. 65 years, complained of attacks of drowsiness and occasional loss of consciousness in the early morning. The symptoms began in the summer of 1943. There was a spontaneous remission, followed by a recurrence, in November of the same year, about 6 weeks before admission to hospital. At that time she was found to have no abnormal physical signs. Her weight in her clothes was then 116 lb. Drowsiness came on during the night and led to semi-coma. On one occasion there was a left extensor plantar response.

The blood sugar was found to be persistently low, particularly in the early morning, by which time the patient would have used up much of the carbohydrate from the last meal (*i.e.* of the previous evening). A diagnosis of hyperinsulinism was made and it was decided to look for an adenoma in the pancreas.

Operation

Laparotomy was performed on 17th January 1945. A small localised tumour was found in the pancreas near the middle of the body. It was shelled out with the aid of scissors. No true capsule was found. No other tumours were found in or near the pancreas.

Naked eye appearance

The tumour was lobulated, roughly globular and firm in consistency. Weight 1·2 g.; size on section 1·5 × 1·0 cm. The whole tumour was embedded in wax after fixation in 10 per cent. formol-saline. No biological assay was undertaken.

Histological examination

Sections were stained with hæmatoxylin and eosin and with Mallory's phosphotungstic acid-hæmatoxylin. The tumour is made up of cells similar to those of the islets of Langerhans. These cells, mostly polyhedral, are

PANCREATIC TUMOUR

FIG. 1.—The dense fibrous tissue stroma of the tumour contains nodules of calcification.   ×65.
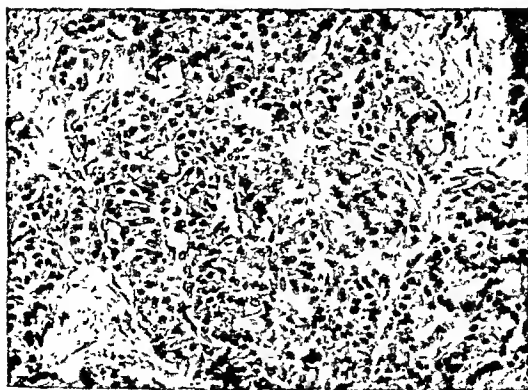
FIG. 2.—"Islet cells" arranged in columns and pseudo-acini.   ×150.

arranged in clusters, columns and pseudo-acini. Some are more columnar in shape and suggest duct epithelium (figs. 1 and 2). In one part of the section there appear to be a few pancreatic acini, the cells of which show purple granules with Mallory's phosphotungstic acid stain. These granules are not seen in the islet cells. The tumour cells are set in a dense fibrous tissue stroma in which there are several areas of calcification. It is not clear whether the apparent capsule encloses the whole of the tumour.

Metabolic studies

On admission, the fasting blood sugar was 41 mg. per cent. (Folin and Wu). In view of this and of the particular periodicity of the drowsiness it was arranged
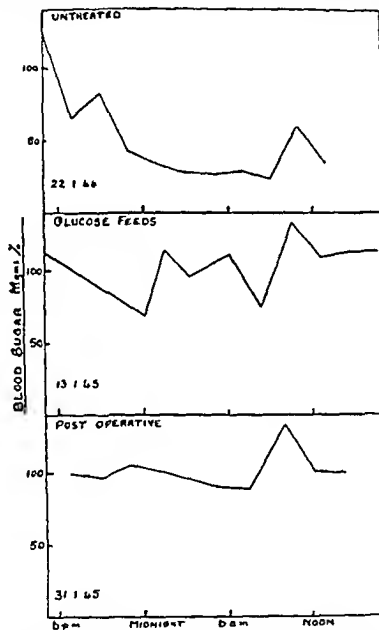
Fig. 3.—Blood glucose levels at intervals of two hours.

Top. Pre-operative : usual diet by day only.
Middle. Pre-operative : usual diet by day augmented by extra feeds at midnight and 3 a.m.
Bottom. Post-operative : usual diet by day only.

to estimate the sugar content in a series of samples taken at 2-3-hour intervals throughout a complete 24 hours. These readings and others taken subsequently are shown in fig. 3. In the first curve, there is a progressive fall in the blood sugar level, commencing before midnight and reaching a minimum of 23 mg. per cent. in the 9 a.m. sample.

It was apparent that the blood sugar level depended almost entirely on the intake of food. Consequently another series of readings was obtained whilst the patient was receiving a normal dietary supplemented by light meals at midnight and 3 a.m. This regime not only showed a more stable level of sugar in the blood but there was also a corresponding lack of drowsiness.

In the immediate post-operative period appreciable lowering of the blood sugar level was anticipated by the giving of meals at night as well as by day. This precautionary measure was continued for three days. The night meals were then discontinued and blood samples were taken at intervals throughout a complete 24 hours. It will be seen (fig. 3) that a sugar level well within normal limits was maintained. Five months later similar readings were obtained, blood sugar levels ranging from 86 to 115 mg. per cent.

Glucose tolerance tests using 50 g. of glucose were also carried out, the first before operation, the second five months later (table).

<div align="center">TABLE</div>

Glucose tolerance before and after removal of pancreatic islet tumour

	Blood sugar (mg. per 100 c.c.)				
	Fasting	¼ hr.	1 hr.	1½ hrs.	2 hrs.
Pre-operative	66	101	121	152	191
Post-operative . . .	97	132	133	123	110

It will be seen that the curve obtained pre-operatively is of the diabetic type whilst the post-operative curve falls within normal limits. Glycosuria was absent throughout each test.

<div align="center">*Commentary*</div>

The morbid anatomy and histology of this type of tumour have already been well described. Occasionally more than one tumour has been present in the same case. Rudd and Walton (1941-42) emphasise the absolute necessity for a thorough search being made in a case of suspected adenoma. These authors, in removing part of the pancreas, accidentally encountered two adenomata at the extreme end of the tail. Tumours have rarely been completely capsulated and frequently it has been difficult to decide whether a given tumour was benign. With the exception of certain undoubtedly malignant tumours (Walker and Boger), the majority have not behaved like malignant growths. Cytologically some of the tumours have been described as "giant islets of Langerhans", but the picture does not always appear to be as simple as this. In the present instance although the cells are mostly of the islet type there is considerable variation. Laidlaw (1938) has made a detailed study of the cytology and reiterates the thesis that pancreatic duct epithelium is or may be totipotent. It behaves in this way in the normal development of the pancreas and may be expected to behave similarly when forming tumours.

In cases where it is not possible to extract insulin from a tumour we can still come near to being sure that some if not all the cells secrete insulin by observing the results of operative removal, as in this case. Barnard (1932) by his study of "A functioning tumour of the islands of Langerhans" has illustrated this point.

It will be seen that the glucose tolerance test in the present case showed a diabetic curve. After removal of the tumour the curve came *within normal*

lumits. This change, or even the presence of the diabetic curve at the outset, is not a constant finding. Whipple and Bauman (1941) reviewed 17 of their cases. They found that the curve was often of the diabetic type and that this tendency might persist after removal of the tumour. In the present case, although there was an initial curve of diabetic type, operation resulted in a return to normal.

Thus the glucose tolerance test, whilst giving interesting results and raising points as yet unexplained, is of no real value in the diagnosis of hyperinsulinism due to tumour. Diagnosis rests rather on the finding, in repeated tests, of abnormally low values for the blood sugar. These results, taken together with coincident attacks of drowsiness which are promptly relieved by the taking of sugar or even an ordinary meal, provide the best method of diagnosis.

Summary

1. A further case of adenoma of the pancreas is reported. Successful operations for tumours of this kind have now been recorded in at least 57 cases.

2. It was not possible to be certain that the tumour was benign.

3. Pre- and post-operative metabolic studies were made, chiefly on the blood sugar. It is suggested that the finding of a persistently low mean blood sugar level is of greater diagnostic value than sugar tolerance curves.

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576 . 8 . 097 . 31 (*Cricetus auratus*) : 576 . 852 . 211

SUSCEPTIBILITY OF THE GOLDEN HAMSTER (*CRICETUS AURATUS*) TO *MYCOBACTERIUM TUBERCULOSIS HOMINIS* AND *BOVIS*

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Experiments on the transmission of *M. tuberculosis* to the golden hamster were recorded by Griffith (1939, 1941), who showed that this species was susceptible to human and bovine strains but that the avian bacillus caused only trivial lesions. Griffith concluded that although the bovine type was the more

virulent, producing extensive caseous or necrotic lesions, human strains were also of high activity. The protocols of his experiments show, however, that relatively high doses were used (1 mg. subcutaneously or 10 mg. by the mouth).

Ungar (1942) noted that after intraperitoneal or intramuscular inoculation of human and bovine strains, hamsters developed lesions similar in character to those seen in guinea-pigs. Virulent human strains in a dose of 0·001 mg. produced enlargement of the regional lymph glands and miliary tubercles of the liver, kidneys and lungs visible to the naked eye in 16-21 days. In spite of the rapid development and miliary character of the lesions, his findings are in harmony with those of Griffith except that the latter did not report any evidence of tuberculous changes in the kidney.

In view of the absence of exact information as to the least number of tubercle bacilli capable of provoking recognisable lesions in the hamster, a series of experiments was undertaken to determine the smallest dose of virulent human and bovine bacilli respectively capable of infecting the hamster and to compare the susceptibility of this species with that of the guinea-pig.

Methods

The strain of *M. bovis* selected was of standard virulence for the rabbit, a dose of 0·001 mg. inducing miliary tuberculosis in about 45 days. The human strain, which had been used extensively on guinea-pigs, was regarded as of normal virulence as judged by the criteria laid down by Griffith.

Cultures were maintained on Herrold's glycerol egg agar. The growth from several tubes of 7-10 days' incubation was weighed, thoroughly triturated in a mortar and diluted in physiological saline solution so that each ml. contained 1 mg. of bacilli (moist weight). Serial dilutions ranging from 1 : 10,000 mg. to 1 : 100,000,000 mg. were inoculated subcutaneously into guinea-pigs. The development of a local lesion was accepted as evidence of the presence of at least 10 viable organisms (Glover, 1944). Hamsters were injected with similar amounts. All survivors were killed at the 180th day.

Results

The details of the experiments are summarised in tables I and II.

M. tuberculosis bovis

It was found that a dose of 1 : 10^4 mg. or more produced a local adenitis which was readily palpable in the guinea-pig in 3-5 weeks, but was less easily detected in hamsters. The disease made rapid progress : in 2 out of 3 hamsters death occurred on the 56th and 90th day respectively with generalised lesions which paralleled quite closely those observed in the guinea-pigs. When the amount of culture was reduced to 1 : 10^5 or 1 : 10^6 mg. extensive lesions were still produced but the period between inoculation and generalisation of infection was increased. Nevertheless widespread tuberculous foci were seen from the 114th day onwards and, when killed, all the animals showed some evidence of spread of infection to the viscera. The end-point in the series for both guinea-pigs and hamsters was 1 : 10^7 mg., since in 4 out of 9 hamsters and 2 out of 5 guinea-pigs macroscopic lesions were demonstrated, although in 2 of the hamsters and in both the guinea-pigs these were strictly localised to the point of inoculation and the contiguous glands. No macroscopic changes resulted from a dose of 1 : 10^8 mg.

A striking feature was the difference in the reaction of the lymph gland system. In the guinea-pig the *classical picture of caseation* of the local lesion and of the lymph glands was invariably observed. As a rule tubercle bacilli

were scanty and were often revealed only after prolonged search. In the hamster, on the other hand, the lymph glands became much hypertrophied and soft in consistency, with a uniform purple-grey colour; caseation was rarely seen. Affected glands were generally teeming with acid fast bacilli.

TABLE I

Results of inoculation of hamsters and guinea-pigs with
M. tuberculosis bovis

Weight of culture inoculated (mg.)	Hamster					Guinea-pig				
	No. of animals inoculated	No. died*	Lesions	No. killed	Lesions	No. of animals inoculated	No. died*	Lesions	No. killed	Lesions
1 : 10⁴	3	2	++++ ++++	1	+++	2	1	++++	1	+++
1 : 10⁵	7	3	++++ ++++ +++	4	++++ ++++ +++ +++	2	2	++++ +++	0	...
1 : 10⁶	9	5	++++ ++++ +++ ++++ +++	4	+++ ++ ++ +	5	2	+++ ++	3	+++ ++ −
1 : 10⁷	9	0	...	9	+++ ++ + + − − − −	5	0	...	5	+ + − − −
1 : 10⁸	5	0	...	5	− − − − −	5	0	...	5	− − − −

* Died before 90th day.

++++ = extensive generalised tuberculosis.   + = local lesions only.
+++ = moderate generalised tuberculosis.   − = no lesions.
++ = tuberculous regional glands.

M. tuberculosis hominis

The human strain also produced a generalised progressive tuberculosis when injected in doses of 1 : 10⁴ and 1 : 10⁵ mg. The course of the disease was, however, more protracted than with the bovine strain but no material difference was detected as between guinea-pigs and hamsters. With a dose of 1 : 10⁴ mg., 2 out of 3 hamsters died at 98 and 106 days respectively with extensive lesions in the spleen and discrete tubercles in the lungs. No deaths occurred before the 180th day in animals receiving the smaller doses (table II). In this series the hamster seemed to be slightly more sensitive than the guinea-pig, since 2 out of 3 showed lesions at 1 : 10⁶ level and 1 out of 3 reacted to 1 : 10⁷ mg.,

whereas only 1 out of 3 guinea-pigs responded, and very mildly at that, to a dose of $1 : 10^6$ mg. and none at $1 : 10^7$ mg.

TABLE II

Results of inoculation of hamsters and guinea-pigs with M. tuberculosis hominis

Weight of culture inoculated (mg.)	Hamster					Guinea-pig				
	No. of animals inoculated	No. died	Lesions	No. killed	Lesions	No. of animals inoculated	No. died	Lesions	No. killed	Lesio
$1 : 10^4$	3	2	+++ ++	1	+	3	1	+++	2	++ ++
$1 : 10^5$	3	0	...	3	++ + −	3	0	...	3	++ ++ −
$1 : 10^6$	3	0	...	3	++ ++ −	3	0	...	3	+ − −
$1 : 10^7$	3	0	...	3	+ − −	3	0	...	3	− −
$1 : 10^8$	3	0	...	3	− − −	3	0	...	3	− −

With regard to the character of the visceral lesions, attention may be drawn to the difference in the hepatic reaction. In guinea-pigs in which generalisation had occurred, multiple macroscopic foci were not infrequent, whereas in the hamster it was difficult to detect liver lesions macroscopically, even in animals in which the spleen and lungs were severely affected with frank tubercles. The reaction assumed the form of a fine widespread focal response, the tuberculous nature of which was confirmed microscopically. No macroscopic kidney lesions were seen except in one animal which received the largest dose of the bovine strain $(1 : 10^4$ mg.).

Summary

Experiments with graded doses of cultures showed that the golden hamster is as susceptible as the guinea-pig to the subcutaneous inoculation of the human and bovine types of *M. tuberculosis*. In each species the minimal infective dose of a fully virulent bovine strain is 1 ml. of a suspension containing $1 : 10^7$ mg. bacilli (moist weight) : the corresponding dose of the human type is $1 : 10^6$ mg.

In the hamster, caseous lesions are not common. A striking feature is a proliferative adenitis in which the glands are teeming with acid-fast bacilli.

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THE EFFECTS OF (a) FREEZE DRYING AND (b) LOW TEMPERATURE
ON THE VIABILITY OF *MYCOBACTERIUM TUBERCULOSIS*

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In a recent series of experiments it was necessary to infect successive groups
of animals with small numbers of viable *M tuberculosis* It is obvious that the
preparation of a fresh suspension for each group is liable to introduce errors
arising from (1) differences in the relation of the weight of a culture to the
number of bacilli, depending upon fluctuations in its moisture content,
(2) progressive loss in virulence on subcultivation and (3) variations in the
proportion of living and dead bacilli in the total culture These difficulties
could be avoided if it were possible to preserve a large batch of bacterial
suspension for long periods without material loss of activity

Conservation of suspensions of virulent tubercle bacilli has been reported
by various authors The methods employed have comprised evacuation of
ampoules and replacement of air by an inert gas (Potter, 1935, 1939), drying
in vacuo on sterile filter paper (Harris and Lango, 1932 33), and rapid desiccation
over P_4O_5 (Cohn, 1939, Darricarrero and Prado, 1940) It has also been shown
that the organisms resist low temperatures Thus Gloyne (1927 28) reported
survival on ice for 12 weeks (not an end point) and Boquet (1943) noted that
at 1° C living bacilli were still obtained after 424 days Kyes and Potter
(1939) observed that freezing in liquid air at −190° C followed by rapid
thawing at 40° C did not affect viability No record has been found of the
behaviour of tubercle bacilli when subjected to modern methods of drying from
the frozen state

In most of the observations cited it is remarkable that little information
has been given as to the proportion of bacilli which survived for any given
period of storage

The work described in this paper was undertaken with the object of
ascertaining the extent of the resistance of *M tuberculosis* to (a) freeze drying
and (b) storage at a low temperature Experiments have been carried out
with the strains of *M tuberculosis bovis* and *M tuberculosis hominis* described
in the previous paper (Glover, 1946)

METHODS

Freeze drying

In the present experiments the apparatus devised by Knox (1939) was
used As Knox has given full account of the precautions which should be
observed in drying pathological products so as to avoid undue loss in potency,
no account of the manipulations need be given

The effect of three substrates on the viability of the tubercle bacillus was
studied Suspensions containing 1 mg per 1 ml were made in distilled water,
physiological saline solution and inactivated bovine serum respectively In
each of these fluids a uniform turbidity without any evidence of coarse particles
was produced Smears showed a satisfactory dispersal of the bacilli with many
single or paired elements and occasional groups of 6 10 bacilli The saline and
serum suspensions exhibited no appreciable alteration after several hours at
room temperature, but the organisms in distilled water tended to aggregate

into fine clumps which could not be readily dispersed. Each suspension was transferred to hard glass tubes (1 ml. per tube) and freeze-dried. One ampoule from each batch was tested immediately after drying and the rest were stored at a temperature of $-4°$ C. The number of viable bacilli which remained was checked at intervals by transferring the contents of an ampoule to a small mortar and adding 5 ml. of distilled water drop by drop to form an even suspension. An equal amount of distilled water was used to wash out the ampoule. The two suspensions when mixed contained 0·1 mg. bacillary bodies per ml.

Conservation at low temperatures

Duplicate samples of the saline suspensions prepared as described were distributed in small ampoules which after sealing were immersed in alcohol maintained at a temperature of $-76°$ C. by solid CO_2. The alcohol was contained in large thermos flasks to which CO_2 was added daily. When required for a test the frozen material was rapidly thawed at a temperature of $37°$ C. and diluted so that each ml. contained 0·1 mg. bacilli.

Serial tenfold dilutions (1 ml.) of reconstituted dried samples and of frozen samples ranging from $1:10^4$ to $1:10^8$ mg. were inoculated into guinea-pigs and hamsters. In view of the results recorded in the previous paper (Glover) on the high sensitivity of hamsters to both human and bovine strains it seemed legitimate to combine the results. In the tables, therefore, the denominators in the fractions refer to the joint guinea-pig and hamster experiments. Tubes of egg-yolk agar were also seeded with 0·02 ml. amounts. In the case of the animal tests all survivors were killed at about the fourth month. Macroscopic evidence of tuberculous foci at the point of inoculation with spread to the contiguous lymph glands was accepted as evidence that the inoculum contained at least 10 living bacilli. Cultures which showed no visible growth were discarded after 10 weeks' incubation.

RESULTS

The results of tests made at various intervals are summarised in tables I and II. In table I the number of bovine organisms as revealed by the biological test was determined at 0, 7, 28, 87 and 180 days respectively after storage at $-76°$ C. and the results compared with freeze-dried material. The viability end-point before storage lay between $1:10^6$ and $1:10^7$ mg. In the case of the low temperature method no significant difference in the number of virulent organisms was detectable over a period of six months. With the dried material, on the other hand, a drop of at least 100-fold occurred immediately. No living bacilli were recoverable at a dilution of $1:10^4$. There was no further decline, however, at the end of six months. The superiority of the low temperature method is thus unmistakable. The type of substrate used for suspending the bacilli before conservation seemed to be immaterial.

The application of the same methods to the human type yielded results in harmony with those obtained with the bovine type. Before storage the strain was active at a dilution of $1:10^6$ mg.: the end-point remained at the same level after 96 days at $-76°$ C. The freeze-dried material on the other hand was completely inactive in the lowest dilution tested, namely $1:10^2$ mg.

Cultural examination

The results of the biological test were checked by submitting the suspensions to cultural examination. An estimation of the total population (living and dead) obtained by direct count under dark-ground illumination showed approximately 1,300,000 bacilli per 0·02 ml. of a suspension containing

1/10th mg. per ml. Serial dilutions were then made of which 0·02 ml. was transferred to each of several tubes of glycerol egg agar medium. The total

TABLE I

Combined results of the inoculation of guinea-pigs and hamsters with M. tuber-culosis bovis (a) after storing at −76° C. and (b) after freeze-drying

Method of preservation	Dose in mg.	No. of days of storage or of freeze-drying							
		0	7			28		87	180
Storage at −76° C.	1 : 10⁴	3/3	3/3			3/3		3/3	3/3
	1 : 10⁵	3/3	3/3			3/3		3/3	3/3
	1 : 10⁶	3/4	1/4			1/4		2/4	2/4
	1 : 10⁷	1/4	0/4			0/4		0/4	0/4
	1 : 10⁸	0/4	0/4			0/4		0/4	0/4
			Dist. water	Saline	Serum	Saline	Serum		Saline
Freeze-drying	1 : 10²	...	3/3	3/3	3/3	3/3	3/3	...	2/4
	1 : 10³	...	3/4	2/3	2/4	2/4	2/4	...	2/4
	1 : 10⁴	...	0/4	0/4	1/4	0/4	1/4	...	0/3
	1 : 10⁵	3/3	0/4	0/4	0/4	0/4	0/4	...	0/3
	1 : 10⁶	3/3	...	...	...	...	...	...	...
	1 : 10⁷	1/3	...	...	...	...	...	...	...
	1 : 10⁸	0/3	...	...	...	...	...	...	...

Fractions indicate numbers of animals which proved to be infected out of the 3 or 4 inoculated.

number of bacilli contained in the inoculum is given in column 2 of table II. The third column shows the estimated number of viable bacilli present in the same dilutions based on the biological test (see table I). Columns 4-6 indicate

TABLE II

Recovery of M. tuberculosis bovis by cultural means from material (a) preserved at low temperature, (b) freeze-dried

Weight of bacilli in 0 02 ml. (mg.)	Estimation of bacilli		Average number of colonies (3 plates)		
	Direct count	Biological test	Before treatment	−76° C. (6 mths.)	Freeze-dried (24 hrs.)
1 : 10	1,300,000	100,000	∞	∞	About 200
1 : 10⁻²	130,000	10,000	∞	∞	20
1 : 10⁻³	13,000	1000	76	44	0
1 : 10⁻⁴	1300	100	10	13	0
1 : 10⁻⁵	130	10	4	2	0

the average number of colonies obtained by the cultural method after six weeks' incubation. These results confirm the biological tests in showing that suspensions stored at −76° C. sustained no appreciable loss in activity after a period of 6 months, whilst the freeze-drying method was responsible for an immediate reduction in the number of living organisms. It will also be noted that, according to the biological test, approximately 10 per cent. of the total bacillary population was composed of living organisms, whereas the cultural method picked out not more than 1 per cent.

Conclusions

1. Human and bovine strains of *M. tuberculosis* suspended in distilled water, normal physiological saline solution or inactivated bovine serum were preserved (*a*) at a temperature of $-76°$ C. and (*b*) by freeze-drying.

2. The number of viable bacilli in ten-fold dilutions was ascertained before treatment and again at varying intervals up to 180 days by the inoculation of guinea-pigs and hamsters and by cultural methods.

3. Suspensions stored at low temperature showed no appreciable loss after 180 days. Freeze-dried material, however, sustained an immediate fall in activity, estimated at 100- to 1000-fold ; thereafter, the dried material remained stable.

4. Bacterial suspensions preserved at $-76°$ C. can be used with advantage in experiments where it is necessary to inoculate small numbers of living bacilli, and to obtain reproducible results.

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PLATE XIII

OBITUARY NOTICES OF DECEASED MEMBERS

John Charles Grant Ledingham

Born 19th May 1875. Died 4th October 1944

(Plate XIII)

John Charles Grant Ledingham was born on 19th May 1875, at Boyndie, Banffshire, where his father, the Rev. James Ledingham, M.A., was minister of the Parish. His mother was Isabella Gardiner, daughter of the Rev. James Gardiner, M.A. He was educated at Boyndie Public School, Banff Academy and the University of Aberdeen. His undergraduate career in Arts from October 1891 to March 1895 was a brilliant one, for he distinguished himself both in classics and in science and graduated M.A. with first-class honours in mathematics and natural philosophy, gaining the Simpson mathematical prize and sharing the Neil Arnott prize in experimental physics. University honours are sometimes of little significance but this was not so with Ledingham, for they were signposts to future achievement. He devoted the next two years to further study and in October 1897 he decided to qualify in medicine. He won high honours in anatomy and gained the Fife Jamieson and Struthers gold medals for proficiency in the subject. In 1900 he took the B.Sc. degree with distinction in mathematics, physics, anatomy and anthropology and in 1910 proceeded to the D.Sc. degree. He graduated M.B. Ch.B. with honours in 1902 and, having been awarded the Anderson Travelling Scholarship, went to Leipzig in September of the same year to study pathology under Marchand with a view to a research career. In 1903-04 he worked in the department of pathology under Hamilton at Aberdeen University and from September 1904 to August 1905 he continued his bacteriological and immunological studies in Bulloch's laboratories at the London Hospital.

Ledingham's association with the Lister Institute began in August 1905, when he was appointed assistant bacteriologist in the serum department at Elstree under George Dean. In 1906 he was transferred to the main Institute in London, together with Dean, who was appointed chief bacteriologist. Dean was elected to the chair of pathology at Aberdeen in 1908, and Ledingham took his place in December of that year as chief of the bacteriological department. He retained this post when he was appointed to succeed Sir Charles Martin in January 1931 as director of the Institute.

He retired from the directorship in March 1943 and died on 4th October 1944.

During the war of 1914-18 he was appointed a member of the Medical Advisory Committee in the Mediterranean area in December 1915 and served abroad with the rank of lieutenant-colonel, R.A.M.C. In August 1917 he became consulting bacteriologist to the Forces in Mesopotamia—a country he had already visited as a member of the Advisory Committee in September 1916, when he made a tour of inspection lasting about six months. He returned to the Lister Institute from Baghdad in May 1919.

In the period between the wars Ledingham's life was filled to the brim with a variety of activities and yet he found time to publish much original work. During the twelve years of his directorship of the Institute he carried the full burden of administration. This, however, brought him the satisfaction of observing the growth of the various research departments and the early stages of important contributions to knowledge in the fields of bacterial and protein chemistry, virus diseases and the employment of efficient bacterial antigens as prophylactic agents.

In 1920 he took part in establishing the National Collection of Type Cultures, which was founded jointly by the Medical Research Council and the Lister Institute. Ledingham, with the aid of St John-Brooks and Miss Rhodes, organised the department and acted as its director during the next ten years. He took steps to create a department of bio-physics through the provision by the Rockefeller Foundation of Svedberg ultra-centrifuges and electrophoresis equipment. On 29th September 1936 Professor Svedberg was present at a demonstration of the apparatus in the biophysical laboratory which had been specially built to house it ; the centrifugal machines were constructed in the workshops of the University of Upsala and have given valuable service.

A considerable portion of Ledingham's time was spent in the work of expert committees, in particular those appointed by the Medical Research Council, the old Local Government Board, the Ministry of Health, the London County Council, the National Radium Commission, the British Empire Cancer Campaign and the Bureau of Hygiene ; he also served on the scientific advisory committees of the Beit and Tata Foundations. He was for a time chairman of the Tropical Diseases Committee of the Royal Society and of the Tropical Medical Research Committee of the Medical Research Council. After the outbreak of war in 1939 he became a member of several important committees concerned with problems arising out of the emergency such as the War Wounds Committee.

In addition to his steady output of scientific papers Ledingham wrote a number of useful reviews of subjects within his special knowledge. In 1912 he joined with Arkwright in writing a monograph on the carrier problem in infectious diseases. He was one of.

the team of contributors to the treatise on diphtheria published in 1923 by the Medical Research Council, and his extensive knowledge of microbiology was freely put at the disposal of the Council in the preparation of their *System of bacteriology* (1929-1931). With Fildes he was an associate editor of the *System* and he contributed the chapters on natural immunity, tularæmia and (with Schütze) the production of active immunity; he also collaborated with Gye in an introductory survey to the volume on viruses and virus diseases. He gave the Harben Lectures for 1925 and dealt with the problems of natural immunity, the carrier problem in disease and the relation of variola to vaccinia. In his Herter Lectures at the Johns Hopkins University, Baltimore, in December 1934 he surveyed the problems presented by virus agents, especially their affinities for the tissues of the host, their immunological aspects and the ætiological significance of the associated elementary bodies.

Ledingham's work met with well merited recognition. He received the C.M.G. in 1918 for his war services and was elected F.R.S. in 1921, serving later on its council. In 1924 he was elected F.R.C.P. London and later served on the council of the College. He received the LL.D. of Aberdeen University in 1935 and he possessed the honorary degrees of Sc.D. Dublin and D.Sc. Leeds; he was knighted in 1937. In 1920 he was given the title of professor of bacteriology in the University of London and in 1944 that of emeritus professor. He served as a member of council of the Lister Institute from 1931 until his death and was a member of the Medical Research Council from 1934 to 1938. In 1938 he was elected a member of the Athenaeum under rule II of the Club, which empowers the election of persons of distinguished eminence in science, literature or the arts, or in recognition of their public services.

On his retirement the governing body of the Lister Institute put on record their tribute to the devoted services he had given without stint to the Institute during his long association with it. Later, he gladly availed himself of the facilities offered to him by his friend, Dr W. E. Gye, for continuing his researches in the laboratories of the Imperial Cancer Research Fund at Mill Hill.

Ledingham married, in 1913, Barbara, daughter of David Fowler of Broomieknowe, Midlothian, who survives him with a son and daughter. His former colleagues and many home and foreign guests of the Lister Institute have reason to remember the kindly hospitality extended to them by Sir John and Lady Ledingham.

His end was unexpected, for he had not been conscious of any weakness, and the tranquil manner of his passing must have seemed to his friends the final boon granted to a life which had fully earned its rest.

In spite of the many calls made upon him in the course of his advisory and administrative work, Ledingham, throughout his

scientific career of nearly forty years, was himself an active experimentalist. His contributions to science give evidence of unusually wide interests, for they include studies in bacteriology, pathology, hæmatology and immunology, and, in particular, researches on virus agents; moreover, he kept constantly in mind the application of scientific knowledge to the prevention of disease.

His activities fall into three periods: the years before the war of 1914-18, the war period itself, and the subsequent years ending in his retirement and death. Two main lines of inquiry engaged his attention in the early years, namely serological studies concerned chiefly with the mechanism of phagocytosis, and the bacteriological and epidemiological aspects of the problem of the typhoid carrier. His experience on active service during the first world war familiarised him with those diseases which were prevalent among the Forces in the Middle East—the enteric group, dysentery, cerebrospinal meningitis and typhus fever. In the post-war period he was chiefly occupied with studies on viruses and virus diseases.

His bent towards research showed itself while he was a medical student, for Prof. Alexander Low (*Aberdeen University Review*, 1944, xxx, 333) tells us that he was one of the founders of the still flourishing Aberdeen University Anatomical and Anthropological Society, inaugurated on 20th June 1899, with Ledingham as its first recording secretary. At a meeting on 6th July 1900 he read a paper on fingerprints (*Proc. Anat. and Anthropol. Soc. (Univ. of Aberd.)*, 1900-02, pp. 13-17), based on the examination of 392 subjects. His correspondence as secretary brought letters of encouragement to the new society from notable anthropologists of the day, including Sir John Evans, Francis Galton, Alexander Macalister, Sir William Turner and E. B. Tylor.

His first important contribution was his account with Marchand in 1904 of a puzzling fatal disease in a German who had returned from the Boxer Campaign in China; histological examination of the spleen, liver, bone-marrow and lymph glands revealed the presence of Leishman-Donovan bodies. This case proved the existence, hitherto unsuspected, of kala-azar in China not long after Leishman had published his account of the parasite. Two papers based on work done during the year spent in the serum department at Elstree and published in 1907 deserve mention. One deals with the leucocytic reaction which occurs during immunisation of the horse and goat with diphtheria toxin. He concluded that doses of toxin sufficiently large to give rise to œdematous local swellings cause a leucocytic reaction of the polynuclear type but that these effects are not necessarily accompanied by increased production of antitoxin. The other paper gives an account of the relation between the antitoxin response and the globulin content of the serum during diphtheria immunisation. A horse whose serum had an unusually high initial globulin content showed no tendency for it to increase during immunisation and gave

a poor antitoxin response, whereas another horse with a significantly lower initial globulin content showed, as Hiss and Atkinson had also found, a progressive increase in the globulins during immunisation and yielded a high-titre antitoxic serum. Ledingham believed that these results indicated a real correlation between an increase in the serum globulins and the antitoxin response. About this time, the Plague Commission in Bombay were investigating the possible significance of chronic rat plague in keeping alight the infection in the inter-epizootic and inter-epidemic period. Ledingham made a histological study of the spleen and liver of 13 cases of natural rat plague collected by the Commission and was of the opinion that the reaction of the tissues against the invading bacteria in some of the sections might readily have proceeded to the complete encapsulation of abscess areas so as to bring about a more or less chronic condition.

Ledingham's studies on phagocytosis (1908) showed that the intake at 18° C. is only one-fifth to one-quarter of the intake at 37° C., and that this result is due to opsonisation of the bacteria being less at the lower than at the higher temperature. With the same degree of opsonin fixation the phagocytic energy of the leucocyte is independent of the temperature within a wide range. His paper (1912) on the mechanism of phagocytosis from the adsorption point of view is an impressive piece of work. The experiments were well designed and gave results which, judged by the accompanying mathematical analysis, could only have been obtained from a remarkably accurate technique and an unbiassed computation of the numerical data from which the phagocytic indices were derived. The discussion of the physical aspects of phagocytosis based on his own observations and those of other workers reaches a high level of well founded argument. Ledingham concluded that the removal of opsonin by a bacillary suspension follows the course of an adsorption process as evidenced by the numerical relations which he found to subsist between bound and free opsonin in equilibrium, and that the phagocytic intake of sensitised or partly sensitised bacteria suspended in normal saline also takes the course of an adsorption process. In the same year there appeared the paper by Ledingham and H. R. Dean on the action of the complement fractions in a tropin-*B. typhosus* system, together with comparative hæmolytic experiments. Although this investigation was beset with variable complicating factors, the results are clearly presented and the nature of the difficulties indicated in a convincing way. If the reader of this paper gives heed to the complexity of the reagents—tropin, mid-piece and end-piece of guinea-pig complement and the phagocytic system—he may well feel surprise that the conclusions are so clearly defined. In the course of this work Ledingham and his co-author confirmed the fact that guinea-pig complement can greatly enhance phagocytosis when typhoid bacilli are sensitised by inactivated typhoid immune serum (tropin), since the combined effect is considerably greater than the

sum of the two separate effects, a type of result for which the term " synergistic " is now used. They tested the relative action in the phagocytic system of each of two fractions of fresh guinea-pig serum (complement), namely mid-piece (euglobulin) and end-piece (the remainder of the serum proteins). The mid-piece was found to be frequently inhibitory to phagocytosis, probably by partial adsorption of the immune end-piece—an effect which would prevent the bacilli from being efficiently sensitised by the tropin. The authors refer this result to the category of inhibition phenomena met with in colloidal chemistry, and it is probable that the physical state of the euglobulin molecules, that is, the degree of their dispersion or aggregation, was the determining factor in the discrepant experimental results.

About this time W. J. Penfold had carried out experiments in the bacteriological department of the Institute to determine the influence exerted on the lag phase in bacterial growth by variations in the initial seeding. Ledingham collaborated with him in a mathematical analysis of his numerical data. The term " lag " is defined as the period which elapses between the time of seeding and the point at which the generation time becomes minimal, and the authors found that during this period growth proceeds in an orderly fashion according to a definite law, that is to say, the generation time steadily diminishes until the minimum is reached.

In the period before the outbreak of war in 1914 Ledingham made a thorough study of the role of the typhoid carrier in spreading the disease and was among the first to direct the attention of the medical profession in Britain to the subject. He joined with his brother, the late Dr Alexander Ledingham, Medical Officer of Health of the County of Banff, in investigating small outbreaks of typhoid fever which had occurred in a mental hospital over a period of at least fourteen or fifteen years ; 3 carriers were discovered among 90 females examined. Ledingham wrote an excellent report to the Local Government Board (1910) on the problem of the typhoid carrier, in which he gave a comprehensive account of existing knowledge.

The pathogenesis of diseases of the blood was a field of inquiry which never failed to attract Ledingham. He felt that the subject had been dominated too long by intensive morphological studies and, although he did not deny the importance of this aspect of the problem, he was convinced of the need for experimental research into these obscure conditions. In 1914 he gave proof of his contention in his paper on purpura produced in animals by administering an anti-blood-platelet serum. The object of the work, when first undertaken, was to determine whether phagocyted blood platelets might simulate certain forms of cell-inclusion. He found that, when an anti-guinea-pig platelet serum prepared in rabbits is injected into the guinea-pig, a condition is produced which resembles purpura hæmorrhagica and presents other features associated with the hæmorrhagic diathesis.

He followed up the inquiry with Bedson (1915) and with Woodcock (1921).

During the last twenty years of his life Ledingham's chief interests in experimental pathology were centred in the problems of viruses and the diseases they cause. His first paper on the subject appeared in 1924 and in it he described the histology of the vaccinial lesions which followed injection of the virus into the rabbit by different routes and, in particular, the changes in the skin. He concluded that the lesion is essentially an acute infective granuloma in which the reaction of the reticulo-endothelial system of cells is the main feature : he could find no evidence for the statement that vaccinia virus possesses an elective affinity for epiblastic tissues. He appears, however, to have underestimated the importance of the epidermal reaction, because work carried out in 1938 by his colleague Amies at the Lister Institute with pure suspensions of elementary bodies confirmed the earlier work on the epiblastic response to vaccinia virus. With McClean (1928) he studied the propagation of vaccinia virus in the dermis of the rabbit. A testis-grown and a " culture "-grown strain of virus, both of which were bacteria-free, were used, and it was found that adaptation of the rabbit testis strain to growth in the dermis of the rabbit was easily obtained, with its potency rising to a high level. But the dermal passages brought about a loss of proliferating power on scarified skin areas for which no explanation was apparent.

Ledingham, with Morgan and Petrie (1931), carried out experiments on the potency and distribution in the serum proteins of anti-viral body obtained by immunising the horse with vaccinia virus. The results showed that the euglobulin contained the antibody in its highest concentration but that its absolute amount was less than that which was present in the pseudoglobulin fraction. The serum, when given simultaneously with the virus, was highly effective in controlling an experimental infection of the rabbit with strains of cutaneous and testicular origin.

Ledingham began to focus his attention on the elementary bodies of vaccinia and fowlpox in 1925, while occupied with various studies on the viruses of these infections. His first note on the subject appeared in 1931 in this *Journal*, when he recorded details of a demonstration he gave of the elementary bodies of vaccinia (Paschen, 1906) and of fowlpox (Borrel, 1904). In a later paper he discussed the significance of these minute bodies and stated that he had succeeded in demonstrating them in stained deposits from potent bacteria-free filtrates of the vaccinia and fowlpox virus after centrifuging them at high speed. He spent five years in fruitless attempts to obtain relatively pure suspensions but at last succeeded by making use of a technique which consists in the trituration of material taken from early lesions, treatment with ether, high-speed centrifuging, and fractional centrifuging of the minute deposits. These finally yield

a suspension which can be specifically agglutinated, a result which he regarded as supporting the belief that the elementary body is the actual infecting agent. Eagles and Ledingham (1932) followed this line of enquiry, which was based on the methods of Ward (1929) and Tang (1930), and showed that by high-speed centrifugalisation—up to 14,000 *r.p.m.*—Berkefeld filtrates of vaccinia virus are depleted almost entirely of their virus content ; the bulk of the virus, consisting of Paschen bodies, is recoverable from the deposit. Ledingham also contributed to this *Journal* two papers (1932 and 1933) on the development of agglutinins for elementary bodies in experimental vaccinia and fowlpox.

In 1935 Ledingham collaborated with Gye in an important research on the nature of the filterable tumour-exciting agent in avian sarcomata. The stained deposits obtained after high-speed centrifuging were found to contain enormous numbers of elementary bodies. Serological tests made with the bodies derived from the Rous no. 1 and Fujinami tumours were found to be specific. Agglutinins for the corresponding elementary bodies were demonstrable in the serum of tumour-bearing fowls. From these experiments the conclusion was drawn that the elementary bodies are extrinsic to the tissues of the fowl.

During the inter-war period, when the study of viruses was Ledingham's main pursuit, he found time to carry out a number of miscellaneous researches. Thus in 1923 he published his observations on the histology of the experimental lesions of tularæmia and on a serological method of diagnosing the disease in man, and in 1924, with F. R. Fraser, he gave an account of the disease as observed in three members of the staff of the Lister Institute who were infected while working with cultures of the causal organism. In 1933 he became interested in the phases of growth in liquid and solid media of the causal agent of pleuropneumonia and of agalactia. His observations were made chiefly from impression preparations of the colonies on serum-agar plates. Ledingham was inclined to place these organisms in the family of *Actinomycetaceæ*. His paper on the subject is a good example of his skilful treatment of a complex problem, in this instance the interpretation of a medley of bizarre, pleomorphic cultural forms whose order of emergence and interrelations are elusive. Ledingham's account of this work shows that his descriptive talent was quite equal to his power of morphological analysis.

The studies on the Foà-Kurloff cell of the guinea-pig give further evidence of his liking for experimental cytology. His first observations were made in 1906 at the London Hospital and the serum department of the Lister Institute, when his attention was directed to " this strange cell ", as he called it—a peculiar form of mononuclear leucocyte containing a mass of finely granular material within a large vacuole. Later workers asserted that the administration of sex hormones increased the number of the Foà-Kurloff bodies both

in castrates and in the new-born. In Ledingham's opinion these observations were statistically weak and so after a lapse of thirty years he made a fresh attack on the problem, and confirmed the dependence of the bodies on the sex organs and their quantitative regulation through sex hormone influences ; he found that œstradiol dipropionate was particularly effective.

Ledingham, as was natural for one who had spent his working life in an Institute founded with the object of furthering the application of newly won knowledge to the practical problems of preventive medicine, took an active interest in the wider aspects of experimental medicine in its relation to the public health. In 1925 he gave an address in which he stated his views on the position of bacteriology in the scheme of public health in Britain and suggested the training of a widespread cadre of bacteriologists for routine duties and research studies throughout the country. The statement embodies principles that have now been adopted to some extent, especially during these recent war years.

In the early 'thirties he made public on several occasions his opinions on the problem of vaccination as a measure of control for both virulent smallpox and alastrim. He insisted that, in order to lessen the risk of nervous sequelæ, vaccination should be done in infancy. He thought that, when mild smallpox is prevalent, vaccination need not be made compulsory, but urged that information on the subject should be broadcast, so that intelligent members of the public might have reasonable ground for accepting the benefits conferred by the practice of vaccination.

In 1939, shortly before the war, Ledingham opened a discussion in Aberdeen on methods of prophylactic immunisation against measles, scarlet fever, diphtheria, whooping cough and influenza, and made a forthright statement of his view that this country, as compared with others, had lagged behind in the application of these procedures. He did not conceal his impatience with the tardy adoption of well tried methods by the authorities and with the lack of adequate means for enlightening those who are responsible for child welfare so that they may understand in some measure the scientific basis of recent methods of prophylaxis. He repeated his plea that a wide extension of laboratory facilities should be made available to public health authorities. Ledingham gave close attention to the problem of providing clean and safe milk for all and devoted much time to furthering this aim. He actively supported the People's League of Health, founded in 1917 by Miss Olga Nethersole, C.B.E., A.R.R.C., and was a member of the Science Council of the League and of its Safe Milk Committee.

In addition to his original contributions to science Ledingham wrote a number of articles summarising the knowledge then existing on subjects upon which he himself had carried out research work. He had a gift for clear presentation and could reduce within reasonable

bounds a mass of observations from various sources and of varying merit so as to give the reader a well balanced account and leave him with the agreeable impression that the subject, far from being complicated, was really a simple one. The articles he wrote for the *System of bacteriology* illustrate his skill in exposition. The best example, perhaps, is the chapter on the many-sided problems of natural immunity, a field of inquiry entangled with the brushwood of contradictory opinions and one that never ceased to fascinate him.

His published work gives evidence of his interest in the history of experimental medicine from the time of Jenner onwards and attests his pride in the accomplishments of the pioneers of the past. In a presidential address given in 1922 he affirmed his belief that knowledge " is best grasped in its historical setting " and insisted on the value of the older work in sharpening the vision of men of science and in fostering an ambition to build truly. He had a keen sense of the continuity of medical research and found pleasure in tracing the early beginnings of scientific principles that are now accepted as fundamental. In 1943 he wrote a brief history of the activities of the Lister Institute since its foundation fifty years ago and in the following year contributed an admirable sketch of the history of bacteriology in Britain for the Medical Bulletin of the British Council.

Ledingham was a man of wide international outlook and did much to promote friendly relations with men of science everywhere, as is shown by the part he took in the three international congresses of microbiology held since the year 1930, when Bordet was elected as the first president. Ledingham was president of the second congress, held in London in 1936, and an honorary president of the third congress which met in New York in 1939, just at the outbreak of the war. The harm done by racial prejudices that hindered the free association of research workers on common ground aroused his indignation and he did not hesitate to make public his views in unequivocal terms. Thus in a letter which appeared in a medical journal only a few months before the war (*Brit. Med. J.*, 1939, i, 697) he put the case against the German policy in words so pointed that they must have stung those to whom they applied, if indeed such men retained any remnant of respect for justice.

When one reflects upon the range of Ledingham's activities one may wonder how it was possible for him to find time for all of them. He was fortunate in several ways. His good natural faculties were strengthened by a sound general education, and an aptitude for the basic sciences of mathematics and physics gave him a secure foundation on which to build when he began his research career in pathology. His mathematical training proved useful both to himself and to his colleagues. The writer was impressed on more than one occasion by the grip with which he fastened on a confused set of quantitative experimental data relating to highly specialised units with which he had little acquaintance and by the skill with which he extracted

from them a clear and logical conclusion. He never lost interest in the classics—a volume of the Loeb edition often formed part of his holiday reading—and he believed that a classical training was an essential part of education, not only on the lower ground of making polysyllabic medical terms easier to the student, but also, on a higher level, for its value in helping the scientific worker to write correct and lucid English.

His memory was exceptionally tenacious and he could track down with unerring certainty a paper in a foreign journal which he had not seen for many years. He had, too, tenacity of purpose, and even after repeated failure he would refuse to be deflected from his aim by difficulties met with in the course of his work. He was an assiduous worker and set no term to his working day. In general his laboratory work was done by means of simple techniques, and yet he quickly realised the importance of utilising modern biophysical apparatus for research into viruses and for many other studies. As a research worker he was an individualist who believed that, whatever planning systems may be adopted in the future, the right kind of researcher should be given full liberty to follow his own impulses. But he welcomed the co-operation of biochemists and biophysicists in solving complex problems which proved refractory to the usual immunological and bacteriological methods.

His success as an administrator was due to his ability to reach sensible decisions by clearly appreciating the central issues of debatable questions. He was friendly and approachable to all the members of his staff. In discussing their scientific problems with them he made helpful suggestions from his own wide laboratory experience but, on the other hand, he could quench insufficiently supported theories with a cold douche of criticism.

His quiet manner, compounded of modesty and reserve, concealed a strong will. At times an overpowering compulsion of will power drove him to exert himself beyond reasonable physical limits; it seemed then as if he were impelled by the subconscious part of his mind. He held a firm rein on the emotional part of his nature and seemed to cultivate a purely objective mental attitude and yet he was considerate, both in word and in action, to those who looked to him for help.

As might be expected in an eager experimenter, he possessed the detective type of mind, and thrillers, mathematical puzzles and acrostics made a strong appeal to him. After a week of hard work he still had a reserve of mental energy to devote to the solution of an acrostic and he was an active member of the " Lister " acrostic club, which included Harden, Arkwright and Robison. " Retsil " —that is, " Lister " in reverse—the nom-de-plume they adopted, if admissible as a homophone, is certainly evocative of an enigma to be solved. He never seemed to feel the need for mental recuperation but found enjoyment and physical refreshment at the sea or in the

country where, in holiday times, he shared in regular family walks. These were made the occasion for rivalry in detecting and identifying fresh species of wayside plants, with " Hooker " as the sole arbiter in dubious cases ; hence the origin of " the lateral squint ", a phrase which took its place among the family jests. He was a competent gardener and preferred this hobby to any other form of recreation. His love of the country as opposed to the town was deeply felt : he found no pleasure in mingling with the crowd, and thus it was that his stay with Lady Ledingham on the Elstree estate of the Institute during the war years before his retirement was altogether congenial.

Ledingham's reserve made him sparing in giving expression to his sense of comedy but, on the other hand, he never took himself or others too seriously. He certainly could relax as, for example, in his speech at the official banquet of the International Congress for Microbiology in New York—an instance of a " a little judicious levity " even in the shadow of portentous world events.

He obtained and gave pleasure by playing Scottish tunes on his violin or on the piano but was not attracted to the complex forms of music. He had no time to seek for interest in the pictorial arts and he found technical discourses on art and philosophy beyond his range, doubtless because his mind was too firmly attached to tangible concepts for him to grasp speculative ideas. A lack of topographical sense was an unexpected weakness in one so observant and so well trained in space-time relations. He failed completely as a guide in a journey by motor car, even when provided with a map and itinerary, and he soon lost all sense of direction when travelling in the Subway in New York : his uncertainty was not relieved by seeking for guidance from a fellow passenger, for this he could not easily bring himself to do, either from shyness or because he was determined to depend upon his own resources.

The science of microbiology has lost an ardent and versatile investigator and an able exponent of its work and aims, who took pride in its past achievements and never spared himself in promoting its interests. His old friends and colleagues will remember him as a sympathetic personality in whose judgment and integrity they could place full trust.

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Joseph Arthur Arkwright

1864–1944

(PLATE XIV)

IT is unusual for a medical man to specialise in bacteriology late in his professional career, though of those who have done so not a few have achieved eminence. Arkwright, whose death at the age of 81 occurred on 22nd November 1944, after a short illness, belonged to this small and select minority. He had been qualified fifteen years before he commenced the serious study of bacteriology and he achieved great distinction in this science.

Joseph Arthur Arkwright was the youngest of five children of Arthur William Arkwright and Emma, his second cousin, daughter of John Wolley of Briston, Nottinghamshire. His father was a gentleman farmer of Broughton Hall, Askoy, Leicestershire, and it was this estate and its immediate environs which formed the setting of the first ten years of Arkwright's life. When he was only two years old his mother died and for a while he was looked after by an aunt. Later this duty devolved on his eldest sister Helen, for whom Arkwright formed a great affection, and it was from her that he acquired that abiding interest in natural history which was to play such a large part in forming the course of his life. The other important formative influence was undoubtedly tradition, for Arkwright had a number of forebears' who gained distinction on the score either of invention or of scientific achievement. His great-grandfather, Sir Richard Arkwright, was the joint inventor with Hargreaves of the spinning jenny, an invention which revolutionised the cotton industry in Lancashire. Sir Robert and Sir James Wigram, the father and brother of Anne Wigram who married Arkwright's grandfather, the Rev. Joseph Arkwright, were both Fellows of the Royal Society, though it seems probable that this distinction was a reward more for interest in science than for actual scientific achievement. Two of Arkwright's maternal uncles were distinguished naturalists. One, John Wolley, an ornithologist, made an expedition to Iceland with Alfred Newton, F.R.S., to study the great auk which was said to be still extant in that island. John Wolley died before Arkwright was born but his books were inherited by the family and those on zoology, with their pictures of animals, formed the favourite literature of Arkwright's early boyhood. Another uncle, Charles Wolley-Dodd, a remarkable amateur gardener, was an authority on alpine plants, so there was much in the family history to inspire Arkwright with an interest in biology. In 1874 at the age of ten he was sent to a preparatory school at Rottingdean on the Sussex coast near Brighton and three years later he followed his brother Leonard to Wellington. It was here that he decided on medicine as a career and on leaving

Joseph A. Arkwright

Wellington he went to Trinity College, Cambridge, there to study for the natural sciences tripos in biology. He took part I in 1884 and part II two years later, with zoology as his major subject. Amongst his teachers at Cambridge whom Arkwright found particularly stimulating were Adam Sedgwick, Alfred Newton and W. H. Gaskell, and he attributed a considerable influence on his intellectual development here to Parkes Weber, a fellow student one year his senior, in whom he found a congenial spirit. From Cambridge he went to St Bartholomew's Hospital for his clinical training, which he completed in 1889 and graduated M.B., B.Ch. The next four years were spent in house appointments, first as house physician at St Bartholomew's and at the Victoria Hospital for Children in Chelsea, and later as house surgeon at the West London Hospital. In 1893 he married and settled in general practice at Halesowen, an industrial area on the borders of Worcestershire and Staffordshire, where he soon acquired a large practice. Arkwright, from all accounts, was a good doctor. In the study and care of his patients his keen intellect and enquiring mind with its biological training found much that was satisfying. He possessed in addition and in large measure those human attributes so essential to the making of a good general practitioner, and he might well have remained in the practice of medicine had not fate determined otherwise. He was subject to hay fever and asthma—he possessed in fact the allergic diathesis and this betrayed itself in general practice by the development of a hypersensitiveness to antiseptics. This gave rise to an intractable dermatitis of the hands which became such a handicap in his work, particularly midwifery, that in 1903 he relinquished his practice. After a short rest he took a small practice at St Margaret's Bay in Kent, where it was hoped that the sensitisation to antiseptics would be less of a disability since midwifery constituted only a small part of the work. However, the dermatitis of the hands recurred and, what was even more disabling, the hay fever and concomitant asthma, engendered by the pollen from the neighbouring downland. This so incapacitated him that after a year he retired finally from general practice. He moved with his family to the vicinity of London and took an appointment as clinical assistant at the Children's Hospital, Great Ormond Street, with the object eventually of becoming a consultant in pædiatrics. Here, however, he became so interested in bacteriology that he decided on a scientific career and in 1905 he applied to Sir Charles Martin, director of the Lister Institute, to be given the opportunity of working there as an honorary research worker. This application was granted and Arkwright started on his career as a bacteriologist in which he was to gain such distinction. The next year or two were spent in equipping himself for this new work. He had much to learn but he could not have been better placed for the purpose, for the department of bacteriology at the Lister Institute when he joined it had as its

director Professor George Dean, and Ledingham, Boycott and Henderson Smith were members of its staff. Arkwright applied himself to this new task with zeal and so well did he progress that soon he had become a competent bacteriologist and had gained the esteem of his new colleagues. In 1908, three years after his admission to the Lister, he was appointed to the staff as assistant bacteriologist and held this appointment until 1927; after his retirement he still continued to work at the Institute as an honorary member of the staff. During these twenty years Arkwright's unbroken series of valuable researches placed him in the forefront of bacteriologists and brought distinction to the Lister Institute itself. Even after his retirement he continued to do original work, but now much of his time and energy were taken up in directing the work of others. He suffered the fate common to so many of those who achieve prominence in scientific work, his counsels were required in the planning and direction of research, and being an unselfish and public spirited person he acceded readily to calls on his time, so much so that by 1930 he had ceased to work in the laboratory and the whole of his time was taken up with scientific administration.

There is little doubt that in medical research Arkwright found the work for which he was best suited. Even preoccupation with a busy practice could not stifle the urge to investigate, and he found time to place on record some of the observations he had made on cases which came under his care. Perhaps the most valuable of the papers that he published whilst in practice was one concerning jaundice in the new born. He was one of the first to observe that this condition occurred in families and he described the case of one woman who had given birth to fifteen children, fourteen of whom developed jaundice shortly after birth and of whom ten died. Recent work on the Rh factor has provided the answer to this problem, which already forty years ago Arkwright had formulated. Had he remained in practice he would no doubt have continued to contribute to the advancement of medical knowledge, but the adoption of a research career enabled him to give free rein to his bent with immeasurably greater results.

During the years which elapsed between his going to the Lister Institute and the outbreak of the first world war Arkwright worked on problems of diphtheria and cerebrospinal meningitis. He showed clearly the part played by carriers in the spread of diphtheria and drew attention to the great variation in virulence which occurred in different strains of the diphtheria bacillus, showing that this was due to variation in toxigenicity. He also observed that the bacillus showed morphological variation and described the short virulent form which we now know as the gravis type. His work on meningococcal infections was concerned chiefly with defining more precisely the means by which the meningococcus could be differentiated from other Gram-negative diplococci and he anticipated to some extent the

work of Mervyn Gordon by showing that the meningococcus comprised a number of serological types. This work provided the material of a number of papers published between 1907 and 1915; some of it was incorporated in a book, *The carrier problem in infectious disease*, which he produced jointly with his colleague Ledingham in 1912. Valuable though this work was as an addition to our knowledge of these two diseases it had an even greater importance, for Arkwright's study of these two micro-organisms showed him that, contrary to the beliefs which then prevailed, bacteria were not fixed in their characters but might show considerable variation. This view found further support in some observations that he made on a strain of *B. acidi lactici* isolated from the urine of a patient with enlarged prostate (1913-14). This organism produced a variant which differed from the original in that it failed to give rise to the formation of gas from fermentable carbohydrates. The variant remained constant in its behaviour on subculture, but it could be made to revert to the original state by growing it in peptone water containing sodium formate. All these findings so impressed Arkwright that he decided to investigate the phenomenon of bacterial variation, a theme which was to inspire some of his best work. Several workers, amongst them Nicolle (1898), Savage (1901) and later v. Lingelsheim (1913) had encountered occasionally an instability in saline suspension of members of the genus *Bacterium*, a state referred to as spontaneous agglutination. This phenomenon remained unexplained until Arkwright, in 1921, showed that it was due to the formation of a variant characterised by a change in the colonial appearances and in serological reactions in addition to the previously observed instability in saline. He applied the term "rough" to this variant as descriptive of its colonial appearance and to distinguish it from the normal form with its smooth colony, and although he and his colleague Goyle (1924) took a false step when they identified the S & R forms with the O & H forms of Weil & Felix, this mistake was soon corrected (Arkwright, 1927). He further showed (1926) that the R form was lacking not only in virulence but also in immunising properties, and finally demonstrated (1927) that in the case of *Bact. typhosum* the O antigens of the S & R forms were both heat-stable and serologically distinct and that the H antigens were the same in both S & R forms and of no value whatever in the production of immunity. An excellent account of this work and other aspects of bacterial variation was given by him in the chapter devoted to this subject in the *System of bacteriology* of the Medical Research Council, though the article is written with such characteristic modesty that Arkwright's own outstanding contribution to this aspect of bacteriological research is by no means obvious. That he was entitled, however, to speak with authority on this subject was recognised by his being invited to open the discussion on bacterial variation at the Second International Congress of Microbiology held in London in 1936.

At the outbreak of the first world war Arkwright turned his attention to the sporing anaerobic bacilli so as to equip himself for the problems which might be presented by the wounds sustained by our troops fighting over the highly cultivated ground of Western Europe. But before he had proceeded very far with this the war had spread to the Near East, and since there was an urgent demand for bacteriologists in this new theatre of hostilities Arkwright offered his services and was posted in 1915 as pathologist in charge of the laboratory at St George's Hospital, Malta, with the rank of temporary captain in the R.A.M.C., his work on the Clostridia being taken over by Dr Muriel Robertson. In Malta he and his colleague Dr Elizabeth Lepper were fully occupied with the work of a busy routine laboratory and there was little opportunity for research, though he found time to produce papers on convalescent carriers in bacillary dysentery (1916) and on blackwater fever (1918).

After two years' service in the Mediterranean area he was recalled to serve on the Commission appointed by the War Office to study trench fever, a new disease which had appeared amongst the troops on all parts of the Western Front and to a less extent in the Near East. The work of McNee, Renshaw and Brunt (1916) had shown that the disease could be transmitted to human volunteers by means of blood and that the infective agent was in the plasma or serum and associated with the washed corpuscles, but that Berkefeld filtration rendered serum non-infective. These facts were confirmed by the British Commission as well as by the workers of the Commission appointed by the American Red Cross. The British investigators also showed that the vector of the disease was the body louse, thus confirming the suggestion put forward independently in 1926 by McNee, by Hurst and by Korbsch. They further established that the infective agent was in the louse excreta and concluded from their experiments, in which a total of 72 volunteer subjects were used, that the bite of the louse did not convey infection, but that the virus was absorbed through skin damaged by scratching. Arkwright and his colleagues Bacot and Duncan (1919) confirmed the observation of Töpfer and others that lice which had fed on trench fever patients developed large numbers of rickettsiæ in the mid-gut and that these rickettsiæ, as da Rocha-Lima had observed, were to be found lying on the surface of the intestinal epithelium and not inside the cells as in the case of *R. prowazeki*. The name *R. quintana* had been proposed for this new species but doubt still existed as to its causal relation to trench fever, since a very similar form—*R. pediculi*—had been erroneously described as occurring in normal lice. It was the work of Arkwright and his colleagues (Arkwright, Bacot and Duncan, 1919; Arkwright, 1919; Arkwright, 1924) which settled the question by showing that the two rickettsiæ were one and the same and by establishing a constant relation between *R. quintana* and the virus of trench fever.

With the disappearance of trench fever in 1918 this important investigation in which Arkwright had played such an outstanding part came to an end and he turned his attention to other species of rickettsiæ and more particularly to *R. prowazeki*, the causal agent of louse-borne typhus. In this work he still had the collaboration of his entomological colleague Bacot and together they published papers on the rickettsiæ parasitic on the sheep "ked" (1921) and on the bed-bug (1921). In their work on typhus, since the disease no longer occurred in this country, they had recourse to animals infected with human material obtained from Ireland. This imposed two serious handicaps; the disease in both the monkey and the guinea-pig lacked any really characteristic features and the human louse could not be kept alive long enough on a diet of monkey's blood to make it possible to study the development of the rickettsiæ after an infective feed. The latter handicap was overcome to some extent by feeding lice rectally with human blood after infection and by studying the development of the rickettsiæ in the monkey louse. In these ways it was possible to confirm the constant relationship of rickettsiæ and infectivity and to make some observations on the morphology of *R. prowazeki*. But these two drawbacks imposed such a brake on progress that in 1922 they received an invitation from the Egyptian Government to study typhus in Cairo, where the disease was endemic, the invitation was accepted without hesitation. Disaster followed this venture, however, for they had proceeded only a short way with their investigations when both contracted typhus. Bacot succumbed, but Arkwright, although a little the elder, recovered after a very severe illness, a recovery attributable in no small measure to the devoted attention of his wife, who nursed him through his illness. Back in London in the summer of 1922 he resumed work at the Lister, but apart from putting together for publication (1923) the observations he and Bacot had made in Cairo his researches on typhus were ended and he turned to other matters, amongst them virus diseases and in particular foot-and-mouth disease.

In 1920 the Ministry of Agriculture and Fisheries appointed its second scientific committee to study the virus of foot-and-mouth disease. At that time none of the small experimental animals was thought to be susceptible, making it necessary to work with cattle and, out of deference to agricultural opinion, which feared that infection might easily spread from the experimentally infected beasts unless they were kept in very strict isolation, the work was carried out on an obsolete warship and attendant lighter moored in the estuary of the Stour at Harwich: Arkwright was put in charge. Investigation of this disease afloat presented very considerable difficulties and progress was slow. And since, in 1921, when the investigation had only just got under way, the Government was looking round for all possible means of cutting down expenditure, this research venture was closed down and Arkwright returned to duty at

the Lister Institute. It was not long, however, before he was back at work on this problem, for in 1924 the Ministry of Agriculture appointed its third scientific committee to study this disease and Arkwright was chosen as one of its members. Sir Charles Sherrington, the first nominated chairman, resigned owing to ill-health before taking up his duties and was replaced by Sir William Leishman. Leishman died within a year and Sir Charles Martin, who was appointed in his place, held this position until his retirement in 1931 when he was succeeded by Arkwright, who continued to direct the work of this committee up to the time of his death. But his contribution to research on foot-and-mouth disease was not merely that of an adviser. The ban on experiments on land had been lifted and work was not only started at the Ministry's laboratories at New Haw and at their Cattle Testing Station at Pirbright, but a part of the committee's research programme was located at the Lister Institute and placed under Arkwright's direct supervision. The work was no longer confined to experiments on cattle, for in 1920 Waldmann and Pape had demonstrated that, contrary to the earlier work of Loeffler, the guinea-pig could be infected, an observation which Hobmaier confirmed a year later; this fact brought work on foot-and-mouth disease within the scope of a research institute such as the Lister and made the prospects of success much rosier. I had the good fortune to be seconded from the staff of the Lister Institute to work with Arkwright on this problem, the other members of the team being Mrs Gibbs (then Mrs Burbury), Sir Charles Martin's daughter, and H. B. Maitland, and the two years that I served in that capacity proved a most stimulating and enjoyable experience. Arkwright did much more than direct our activities for, until his retirement in 1927, he devoted a considerable part of his time to active participation in the investigations. He was not very able technically, for he was handicapped by a tremor which at times made fine work with his hands extremely difficult. But his broad scientific outlook and wide experience of microbiological research made his counsel invaluable and it was invariably given with such courtesy and consideration that it obtained ready acceptance. The first task of Arkwright's team was to confirm the susceptibility of the guinea-pig and this having been shown to be correct a study of the virus was begun. Amongst the points investigated were the filterability of the virus and its behaviour to heat, drying, ultraviolet light, chemical agents and changes in pH. The susceptibility of a number of rodent species which might play a part in the spread of the disease was explored and the mechanism of the immune state left on recovery from infection was studied. Observations were also made on the multiplicity of serological types in strains of virus isolated from outbreaks of the disease in the country and a beginning was made in an attempt to find some safe means of conferring active immunity. In this latter work the only promising vaccine was one in which the virus had been

inactivated by formalin in low concentration, confirming an observation made by the French investigators Vallée, Carré and Rinjard. In April 1924 the German workers Frosch and Dahmen announced that they had succeeded in cultivating the virus on a simple artificial medium, and it seemed to the Committee that this finding was of such outstanding importance that arrangements were made with Professor Frosch for Arkwright and me to see their work. We went to Berlin in July of that year and spent several days with Dahmen. Since my knowledge of the German language is by no means good I was relying on Arkwright to act as interpreter, but much to my dismay I found on the occasion of our first meeting with Dahmen that Arkwright was just as much at a loss to understand him as I was myself. Fortunately Dahmen and I found in French a common medium of communication and we were thus able to get all the information we required. We left Berlin unconvinced as to the correctness of this claim to have grown the virus and back in this country we were not long in satisfying ourselves that the so-called colonies which the German workers had observed in their cultures were only artefacts which could be produced equally well if the medium was rubbed with a sterile platinum loop or inoculated with a solution of perchloride of mercury. It was characteristic of Arkwright's generosity to his junior colleagues that he only put his name to one of the many papers in which this work was published ; he stood aside in order that they might have the credit for work much of which he had directly inspired. Arkwright's brilliant research work did not of course go unnoticed ; it gained him the esteem of bacteriologists the world over and it also received well merited recognition in his election to the Fellowship of the Royal Society in 1926.

The last fifteen years of Arkwright's life were largely devoted to scientific administration and in this as in all else that he undertook he gave of himself to the full. When the Agricultural Research Council was formed in 1931 Arkwright was chosen as one of its members and he gave invaluable service in this capacity for eleven years. He was chairman of the committee appointed by the A.R.C. to study *Br. abortus* infection so widespread in our dairy herds and he was largely instrumental in founding the Field Research Station at Compton in Berkshire. He was also made chairman of the A.R.C. committee on Johne's disease and reference has already been made to the long and valuable service which he rendered to the Ministry of Agriculture in connection with foot-and-mouth disease, first as a member and later as chairman of the third scientific committee appointed by the Ministry to study this disease.

In 1916 he was elected to the Fellowship of the Royal College of Physicians and he served on its council from 1929 to 1931. He was equally active in the twin field of medical research ; in fact he was a great exponent of the unity of veterinary and human medicine,

taking this theme for his presidential address to the Section of Comparative Medicine of the Royal Society of Medicine in 1932. He was made a member of the Medical Research Council in 1930, retiring after the normal period of four years' service, and when the M.R.C. and A.R.C. jointly appointed a committee to study tuberculosis Arkwright was chosen as chairman. He succeeded Sir David Bruce as the Royal Society's representative on the Governing Body of the Lister Institute, thus helping to shape the destinies of the institute which he had served so long and so well as a member of its staff. It would have been surprising if such a record of service to veterinary and medical research had gone unrecognised by the State and in 1937 Arkwright received a knighthood as some acknowledgment of all this valuable and unselfish work.

Arkwright was a shy and reticent person and this made him a little unapproachable; even after working in close association with him for several years I did not know him really well. But one soon learned that he possessed many very fine qualities. He was utterly sincere in all that he said or did so that one always knew exactly where one stood with him, and another outstanding quality was his modesty, which remained unaltered despite his many successes and achievements. He was kindly and courteous, particularly to his junior colleagues whom he was always prepared to help in the most unselfish manner; their advancement and success seemed to be of greater moment than his own. And he was a most charitable person. I do not recall ever hearing him say anything harsh or unkind about other workers. A well balanced outlook and well informed mind made his conversation interesting and often illuminating, but he did not speak well in public; in fact, unless he had prepared what he had to say, his contributions to scientific discussions were sometimes halting and possibly did not carry the weight to which their factual content entitled them. This lack of fluency and conviction as a speaker also showed itself, though to a less extent, in his committee work, and the fact that he was so much in demand in this respect was a great tribute to his erudition and extensive knowledge.

Apart from his work his principal interests were in things of the countryside. He was a good field naturalist and a keen gardener and when at the Lister Institute I often went with him to the Chelsea Flower Show or to the smaller exhibitions of the Royal Horticultural Society at Vincent Square. Ledingham, another enthusiastic gardener, was usually a member of the party and there was often heated discussion on the merits of different flowers, a subject on which Arkwright held very definite views. He had no use for flowers which were not of a true self colour; the new delphiniums, for example, with their shades of mauve and purple were to him anathema and were rigorously excluded from the beautiful garden at his home in Purley, the care of which gave him so much pleasure and restful recreation.

A simple, kindly, good man and a grand colleague, he was without an enemy and there must be many, like myself, who heard of his death with genuine sorrow. Despite his great age he retained an agility of mind and body which was really remarkable ; it seemed that he would never grow old. Fortunately when the end came it was as the result of a brief illness ; he had remained in harness to the last —a fine record.

In 1893 he married Ruth, second daughter of Joseph William Wilson and Harriet Anne Wilson (*née* Moore) of Elmshurst, Kenley, Surrey. His wife before her marriage was a sister at Arkwright's old hospital, St Bartholomew's, and of their three daughters, all of whom are married, two are members of the medical profession.

S. P. Bedson

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Thomas Henry Belt

Born 19th July 1902. Died 7th March 1945

Thomas Henry Belt, who died on 7th March 1945, at the age of forty-three, was Canadian born of British stock. A " son of the manse ", he was born in Ancaster, Ontario, where his father was an Anglican clergyman. He was a great-grand-nephew of Thomas Belt (1832-1878), mining engineer, geologist and explorer, whose book *The Naturalist in Nicaragua,* published in 1874 and reprinted in Everyman's Library, was described by Charles Darwin as " the best of all natural history journals which have ever been published ". T. H. Belt went to school in Toronto, where he matriculated with first class honours in 1919 and entered the University in 1922. In his fifth year he was awarded the John MacCrae Memorial Scholarship and the following year became president of the undergraduate medical society. He graduated M.D. with honours in 1928, and in 1929 obtained the postgraduate degree of B.Sc. (Med.). After graduation he spent two years as fellow in pathology under the late Oskar Klotz. He was then awarded a travelling fellowship and spent a year in Germany under Aschoff and Fischer-Wasels. On returning to Toronto in 1931, Belt rejoined the Pathological Department and held the post of lecturer in pathology until 1935, when he became pathologist to the Toronto Hospital for Consumptives. From 1934 to 1937 he was coroner's pathologist for the City of Toronto and in 1937 he was appointed senior assistant in morbid anatomy at the British Postgraduate Medical School.

A competent morbid anatomist and histologist, Belt was a keen and shrewd observer and in his morbid anatomical work he had a well balanced sense of proportion, which his experience as a coroner's pathologist had doubtless helped to develop. He was a rapid and able dissector and a clear and logical expositor.

Belt's published research work was contained in some twenty papers which fell mainly into three groups. His earlier papers, on yellow fever, were largely inspired by Klotz and were concerned with the changes in the liver and spleen in that disease and with the identity of the lesions in African and American yellow fever. His second important group of publications was concerned with pulmonary embolism, and here he broke new ground in demonstrating the frequency of this condition in a wide variety of chronic diseases and in showing the origin of these emboli in the leg veins. The pertinacity with which he sought for emboli in routine autopsies and the frequency with which this search was successful and the results displayed at his post-mortem demonstrations at the Postgraduate School, caused the condition to be labelled by his colleagues, in lighter vein, as " Belt's Disease ".

His contributions to silicosis were considerable and must be regarded as of lasting value. It was, in fact, his interest in this condition which led the late E. H. Kettle to desire to have Belt as one of his colleagues at the Postgraduate School. Much of this work was described in a series of papers appearing in the *Journal* over a period of several years. These presented the results of careful and penetrating histological analyses of both human and animal silicotic material, and undoubtedly led to a better understanding of the fundamental pathological process at work in the dust-ridden lung. Prominent among Belt's contributions in this field was his discovery that crystalline particles of silica tended to disappear from the centre of the maturing human silicotic nodule while remaining visible at its periphery. This change of crystalline silica into an occult form has been referred to in the literature as " the Belt phenomenon ". His finding that the silicotic nodules produced experimentally in animals seldom progressed beyond the stage of reticulin formation and never presented the massive collagenous fibrosis of compact structure seen in human silicosis, led him to examine carefully the progress of both animal and human lesions and to look for an apparent arrest of the process in lungs which had been exposed to a highly modified silica hazard such as is seen in coal-miners. This he seemed to have found in the condition known as " dust-reticulation " in colliers. The Medical Research Council's Special Report Series, nos. 243 and 250, contain considerable and well documented papers by Belt on the pathology of coal-miners' lungs and on the tissue reactions produced experimentally by selected dusts from the South Wales coal mines.

Belt was a vigorous personality, humorous and tolerant, a good teacher and a critical observer. His last years were clouded by ill-health and a good deal of unhappiness, but he will be remembered by his friends as the man he was before these shadows fell across his life.

J. H. DIBLE
E. J. KING

Jean Orr-Ewing

Born 28th April 1897. Died 17th November 1944

THE death of Jean Orr-Ewing at the early age of forty-seven has deprived her College and the Oxford Medical School of a most valuable teacher and research worker. Her first contact with Oxford University was in 1916 when she became a student of Lady Margaret Hall and there carried out her preclinical studies with considerable distinction. From Oxford she passed on to St Mary's Hospital, London, for her clinical work, taking the Conjoint Diploma in 1923, and the Oxford B.M., B.Ch. degrees the following year. Several years of scientific work followed, partly in the department of pathology under Professor Dreyer and partly under Professor Peters in the department of biochemistry, leading to publications, chiefly in collaboration, on bacterial growth-promoting factors and on the glucose tolerance of rabbits.

Her election to the Schorstein Research Fellowship in 1929 enabled her to pursue these and other researches, and in the same year she was appointed lecturer in natural science at Lady Margaret Hall. It seems that at this time she acted as tutor to all the Oxford women medical students, of whom there were not yet a great number. Later, in 1938, her College elected her to a tutorial fellowship, which she held till 1943, when growing ill-health forced her to resign. From 1932 to 1939 she also held a tutorship in the Oxford Society of Home Students, now known as St Anne's Society. In spite of heavy teaching duties she always managed to spend time in scientific research on subjects ranging from the serological typing of diphtheria bacilli to penicillin and the bacteriology of war wounds. During the work on diphtheria she was unlucky enough to sustain an accidental diphtherial infection, which left her with a slight permanent weakness of the heart.

All her scientific work was characterised by untiring keenness and searching self-criticism; while the high standard she required of her pupils inspired the strong and scared the indolent into activity.

Perhaps it was the too wide dispersal of her energies that prevented her from making a greater name in any one branch of medical science; for she was, in addition, a prominent member of the Oxford Ornithological Society, a very efficient tennis and hockey player, a great climber of Swiss mountains and Lake-land rocks, and the organising leader of the University Women's Iceland Expedition in 1934.

Although from the time of a serious surgical operation in 1943 she was increasingly aware that her life was threatened, she came back to work as bacteriologist in the Emergency Public Health Laboratory Service at Oxford, and persevered until her worsening condition made it physically impossible to continue. Though her body was visibly breaking the cheerful fortitude of her spirit was, to the end, the admiration and envy of her friends.

A. D. GARDNER

BOOKS RECEIVED

Trauma in internal diseases

By RUDOLF A. STERN. 1945. New York, Grune and Stratton : London, William Heinemann (Medical Books) Ltd. Pp. xviii and 575. 30s.

This is an American revision of the same author's " Über traumatische Entstehung innerer Krankheiten ",' Aufl. 3, Jena, 1930, modified by appropriate topical references to medical and legal practice. Altogether, there are some 2000 citations mainly from German and American sources.

The keynote of the work is expressed best, perhaps, in the discussion of diabetes mellitus (p. 451) : " . . . I would consider a causal relationship of trauma and diabetes as probable if the following postulates are satisfied : 1. Perfect health (in the accepted sense of the word) prior to the accident. 2. Adequacy of the trauma, *i.e.* injury either of the pancreas or of the central nervous system or severe psychical trauma. 3. Development of the diabetes within a few days or at least weeks following the trauma. . . . By adopting these principles, compensability might erroneously be acknowledged in a few cases ; but any other procedure might result in a flagrant injustice to an injured person. I do not need to repeat that in formulating these principles for *practice,* we do not pretend to solve the *scientific* problem of traumatic diabetes ". That is the rub, and it is constantly recurring through the gamut of internal diseases with which the author deals in detail. Nevertheless, a lucid exposition of the principles involved in assessing the *practical* significance of non-perforating trauma in internal diseases is copiously illustrated by critical case reports and legal judgments. Thus, this work should prove a valuable book of reference for medical referees and assessors and, indeed, for all medical witnesses who are concerned with Workmen's Compensation cases. As the author emphasises in the introduction (p. xxii) : " . . . cases which are the subject of expert opinion rarely fulfil those requirements which one demands for a scientifically sound opinion ". This does not mean that the contents of the book are of little or no interest to the scientifically-minded reader. Apart from the intrinsic merit of the review of traumatic diseases (real or alleged) of the cardiovascular system, occupying nearly one-third of the book, the wide cleavage between scientific and legal proof in respect of traumatic ætiology is a matter of constant interest and it is well that pathologists should be aware of it on the brink of a national health service, with or without social insurance.

Injury and death of bacteria by chemical agents

By OTTO RAHN. 1945. Normandy, Missouri : Biodynamica. Pp. 183 ; 34 text figs. $3.60.

In this monograph, dealing with the " injury and death of bacteria by chemical agents ", the author divides his work into three main parts. The first gives a clear account of all the known facts about the death of bacteria and the laws governing it. This can be regarded as a handy and accurate resumé of, as he calls it, the logarithmic order of death of bacteria. The second part deals with chemical disinfectants and their effects on

bacteria. Although there is nothing new in this part, it also is a good record of chemical disinfectants and their actions. The third part of the book is concerned with antiseptics, their method of testing and the factors which govern the action of these bacteriostatic substances.

In dealing with disinfectants and antiseptics the author clearly points out that there is great difficulty in accurately defining what is a disinfectant and what is an antiseptic. He appears to regard disinfectants as being lethal to bacteria whilst antiseptics inhibit their multiplication. The action of disinfectants he considers as something final and irrevocable whilst that of antiseptics is something which can be interrupted. He does indicate that there is considerable divergence of opinion on this latter point, as it is known that antiseptics under certain conditions can act as disinfectants and vice versa. Thus he says " a precise distinction between disinfectants and antiseptics is possible only by choosing an arbitrary time limit and by specifying the conditions ". For assaying disinfectants he uses a comparison based on the phenol coefficient. The efficiency of antiseptics cannot be assessed with this technique ; thus no coefficient can be established for them.

The first two parts of this work may be regarded as a very useful monograph on the causes of death of bacteria by chemical agents ; the third part however does not come up to the same high standard. Although the author deals very accurately with the action of the sulphonamides and related drugs, there are omissions. Little is said about other drugs or their mode of action, or about the drug resistance of bacteria. This is to be expected from any review of work which is still in progress and still as incomplete as the mode of action of bacteriostatic or chemotherapeutic agents. Nevertheless the book can be thoroughly recommended to all who are interested in disinfectants and bacteriostatic agents.

What people are: a study of normal young men

By CLARK W. HEATH, in collaboration with LUCIEN BROUHA, LEWISE W. GREGORY, CARL C. SELTZER, FREDERIC L. WELLS and WILLIAM L. WOODS. 1945. Cambridge, Mass. ; Harvard University Press : London ; Humphrey Milford, Oxford University Press. Pp. xvi and 141 ; 4 plates and 4 text figs. $2.

Having postulated that normality represents a " balanced, harmonious blending of functions that produce good integration ", the author and his co-workers set out to discover the nature of normal young men. Their techniques of investigation include a thorough physical examination, physiological tests of stability, *e.g.* basal metabolism, insulin tolerance and the like, an anthropological survey of body structure, psychological measurement of mental functions, a socio-economic anamnesis and several psychiatric interviews. These methods were applied to a few hundred undergraduates chosen from amongst those showing academic aptitude or promise.

Despite this apparently comprehensive approach, the results of the investigations are nebulous. This is due not simply to the fact that Mr Heath presents his material badly but to his conviction that " normality " can be comprehended by studying various end-products of normal function. He is obsessed with the view that it is undesirable to approach the normal with criteria derived from study of the abnormal, in particular from psycho-pathological data. The absurdity of this position is clearly brought out in his statistical tables. When he comes to classify normal characteristics, the author is compelled to isolate abnormal manifestations under separate headings, *e.g.* " unstable ", " asocial ", " incompletely

integrated ". This of course begs the definition of the " normal young man " and is merely exasperating to any reader familiar with the relation between symptoms of mental disorder and regressions to earlier forms of " normal " function.

But although Mr Heath conspicuously fails to fulfil the promise of his title-page, his very failure serves to call attention to the need for closer co-operation between organic pathologists and psycho-pathologists. Psycho-somatic investigations have been too long in the undisciplined hands of psychologists holding sketchy and tendentious views of organic function. Organic pathologists have an important part to play in psychosomatic research and cannot reasonably justify their aloofness on the ground of ignorance of the elements of clinical psychology. Both disciplines are essential to advances in the debatable ground between the mental and the physical.

EDWARD GLOVER

Hay fever plants

By ROGER P. WODEHOUSE. 1945. Waltham, Mass.; The Chronica Botanica Co. : London ; William Dawson & Sons, Ltd. Pp. xix and 245 ; 73 text figs. $4.75.

This publication is vol. 15 of the " New Series of Plant Science Books ", edited by Frans Verdoorn. It is a description of the hay fever plants : their appearance, distribution, time of flowering and role in hay fever, with special reference to North America, of purpose " to interpret the botanical facts of hay fever in terms of their clinical significance ".

The contents comprise the botany of hay fever, the hay fever plants, gymnosperms, angiosperms, monocotyledons, dicotyledons, regional pollen surveys, glossary, bibliography, and author and subject indices. The author—a well known authority on pollen and allied subjects—gives a competent and comprehensive description of the plants known to cause hay fever. The regional pollen surveys are limited to the United States, Canada and Mexico. The lay-out of the book has a peculiarly amateurish flavour (increased by the decoration of blank spaces in the title-pages with drawings of what at first sight appear to be knobbly cannon balls— presumably magnified pollen grains), and the occurrence of a printer's error in the first sentence of the book is unfortunate. The numerous illustrations are poor and the production generally is much below the high standard that one has grown accustomed to expect of American scientific publications.

The book should prove a useful reference work for the student of hay fever, to whom its appeal will naturally be limited.

Kettle's pathology of tumours

By W. G. BARNARD and A. H. T. ROBB-SMITH. Third edition. 1945. London : H. K. Lewis. Pp. viii and 318 ; 191 text figs. 21s.

The enduring quality of Kettle's book is shown by the fact that a large part of the text and 158 of the 191 illustrations of this third edition are retained from the second edition of twenty years ago. The glazed paper now used yields, with a few exceptions, better reproductions of the illustrations ; the new pictures are mostly photographs, without magnifications or the optical details attached to Kettle's drawings. The volume is laid out as before in three parts, on the general biology and general and special pathology of tumours.

There is, of course, much more to be said now than then on such aspects as heredity and experimental carcinogenesis, and considering the extent

of the field to be covered, the account is very succinct ; Kettle's somewhat detailed account of the transplantation of tumours is now perhaps disproportionately lengthy in its new context. A new section on occupational cancer contains a convenient table of occupational cancers and their experimental equivalents. There is no reference to the work of Warburg and others on the metabolism of tumour tissue. The section on treatment obviously had to go, but it is a pity that the discussion on biopsies could not have been retained.

There is a new section on tumours of nervous tissue, concise and in the main clear, though some of us may fail to be convinced by the explanation of the difference between Schwannoma and neurofibroma. As might be expected, the authors have introduced a pithy account of bronchial carcinoma, and one of reticulosarcoma ; it seems a pity, however, that the familiar lymphosarcoma should have to become the lymphocytic reticulosarcoma. The classification of ovarian tumours has been brought up to date, and almost all the section on ductless glands is new. The term " perithelioma " appears now and then, and is very properly condemned. Might it not now disappear ?

Proof correction has been inadequate ; some of the figures are wrongly orientated and spelling mistakes are too frequent. " Perineureum " and " ondeneureum " each appear twice in five lines (p. 175), " cartilagenous " four times (pp. 216-218), and the adjectival " scirrhus " (pp. 135 and 243) may exacerbate an already notorious student illiteracy. The book, however, remains thoroughly readable, the information is accurate, and it should be popular with medical students.

Clinical pathology

By P. N. PANTON and J. R. MARRACK. Fifth edition, 1945. London : J. & A. Churchill. Pp. x and 450 ; 47 text figs, 40 figs. (38 in colour) on 12 plates. 21s.

Within the limits set by its size (the fifth edition is reduced from 502 to 450 pages) and scope (the whole field of clinical pathology is covered), this book is likely to be valuable to others than the expert in providing reliable information about pathological processes and technique. It is pleasant to read, concise, critical and on the whole accurate. It inevitably suffers from compression but it is difficult to see which section could have been omitted without detracting from its claim to completeness. In the opinion of the reviewer, books containing more detailed information concerning a more limited field are of greater value as works of reference, even in a small laboratory, but where much information in a limited compass is required, this book could hardly be surpassed.

PROCEEDINGS OF THE PATHOLOGICAL SOCIETY OF GREAT BRITAIN AND IRELAND

4th January 1946

The seventieth meeting of the Society was held in the Medical School of the Westminster Hospital, London, on Friday, 4th January 1946.

Communications and demonstrations

The item marked with an asterisk is abstracted below.

A. C. LENDRUM. Pulmonary hæmosiderosis.

R. A. WILLIS. Intra-pericardial teratoma in a child.

L. DMOCHOWSKI and R. J. LUDFORD. Experiments in the treatment of transplantable tumours with stilbœstrol.

A. M. BARRETT and L. B. COLE. Malignant pulmonary hypertension.

I. DONIACH. Combined anterior pituitary necrosis and bilateral symmetrical cortical necrosis of the kidneys following concealed accidental hæmorrhage.

A. ELKELES and L. E. GLYNN. Mitral stenosis associated with parenchymatous ossification in the lungs.

D. M. PRYCE. Dating of lower accessory lung.

R. D. PASSEY. Addison's anæmia and gastric carcinoma.

A. D. TELFORD GOVAN. Observations on acidosis due to ammonium chloride.

R. A. M. CASE. Toxic effects of 2, 2-bis (p-chlorphenyl) 1, 1, 1-trichlorethane (D.D.T.) in man.

A. W. GLEDHILL. Some properties of a thermolabile antigen of *Erysipelothrix rhusiopathiæ*.

E. P. ABRAHAM and E. S. DUTHIE. Effect of the hydrogen-ion concentration of the medium on the activity of penicillin, streptomycin and other chemotherapeutic substances.

D. F. CAPPELL and G. HARVEY-SMITH. Histological sections from sternal puncture biopsy.

A. W. BADENOCH and E. M. DARMADY. Partial occlusion of the renal artery in rabbits and its relation to traumatic uræmia.

A. J. RHODES, S. W. G. HARGROVE and J. H. FODDEN. A case of malignant granuloma of the nose.

A. J. McCALL. A case of cystic pneumatosis of the intestine.

SHEILA CALLENDER and R. R. RACE. Transfusion made difficult.

A. E. MOURANT and R. R. RACE. Charts illustrative of Rh genes, antigens and antibodies.

*J. C. WHITE. An improved method for the histological examination of sternal puncture material.

LUCY D. MEYRICK. Bilateral primary carcinoma of the fallopian tubes.

M. H. SALAMAN. The association with the gonococcus of structures typical of organisms of the pleuropneumonia group.

DOROTHY S. RUSSELL. Rhabdomyosarcoma of petrous bone in a girl of four, with metastasis to lungs and bones.

Abstract

578 . 67 : 611—018 . 46

AN IMPROVED METHOD FOR THE HISTOLOGICAL EXAMINATION OF STERNAL PUNCTURE MATERIAL

J. C. White, J. R. Baker and J. G. Griffin

From the Department of Pathology, The British Postgraduate Medical School

Sternal puncture is now a widely practised procedure which yields most valuable information on the detailed cytology of the bone-marrow, particularly by means of Romanowsky-stained dry films. Histological sections of the bone-marrow during life provide much additional insight into the quantitative and qualitative changes in the myeloid elements in disease, particularly indicating increased or decreased degrees of cellularity which are difficult to gauge from examination of films and total nucleated cell counts alone. Sections may be prepared by suitable manipulation of the mixture of blood and semi-solid cellular material obtained by sternal puncture, or by sternal biopsy, in which the outer plate of the sternum is trephined and the marrow cavity curatted ; the material obtained by the latter procedure will usually require decalcification before it can be stained.

Custer (1933) has described sternal biopsy and gives details for the preparation and interpretation of sections. The method gives an adequate amount of material, which is rather more likely to be representative of the marrow as a whole than the small sample obtained by puncture, but it has the disadvantage of being a more complicated procedure requiring surgical technique, with more disturbance to the patient than sternal puncture. This is a very simple operation and very safe, provided that proper aseptic precautions are observed.

The material obtained is almost invariably free from bony spicules and requires no decalcification, but the marrow fragments are very small and difficult to handle. For the preparation of sections, Davidson (1941) recommends allowing the aspirated material to clot in a watch-glass, removing the clot to a small piece of tissue paper and fixing. Mertens (1945) has recently described a method in which the sample is allowed to clot in the syringe used for aspiration, followed by fixation of the coagulum. A comparison of the results from films and sections has been made by Mulligan (1942).

The following method has been developed as a means whereby both films and sections may be obtained at a single puncture with a minimum of manipulation. It gives excellent material for comparative study.

1. Sternal puncture is performed in the usual way, using a Salah needle. Not more than 0·2 c.c. of marrow is aspirated into a 2 c.c. Record syringe.

2. Films are prepared by carefully expelling small drops of marrow from the syringe on to clean dry slides, sucking back excess of blood and spreading at once, preferably by an assistant. From 12 to 18 films can usually be made and treated by drying in the air and staining with Jenner-Leishman or other suitable stain. Alternatively wet fixation and staining may be used.

3. The material remaining in the syringe (rather less than half) is rapidly expelled from the vertically held syringe into the fixative contained in a 15 c.c. conical centrifuge tube with a moderately wide bottom. The fixative which has been used is a mixture of 9 c.c. of absolute methyl alcohol and 1 c.c. of neutral formol (40 per cent.). Particles of marrow rapidly settle to the bottom, whilst blood clot forms a suspension entangling more marrow.

4. Within a few minutes of taking the sample the tube is centrifuged at 3000 *r.p.m.* for 10 minutes, giving a disc of marrow and clot at the bottom of the tube. Fixation is allowed to proceed for 12 hours.

5. Pour off fixative and replace by two changes of absolute ethyl alcohol for three hours each.

6. Replace with a mixture of equal parts of absolute ethyl alcohol and ether for two hours.

7. Replace by 1 per cent. Necol (1 per cent. Necol 356 A/9 in 50 : 50 absolute ethyl alcohol and ether) or 0·25 per cent. celloidin overnight. At first the tube is gently inclined at intervals to ensure good infiltration.

8. Pour off the Necol and invert the tube, leaving to drain for 3-5 minutes.

9. Cover the marrow with chloroform for 3-5 minutes to harden.

10. Detach the cast of marrow from the bottom of the tube. This was at first effected by a mounted needle and platinum loop, but it has been found that gently warming the bottom of the tube over a Bunsen flame frees the tissue completely. Leave for a further five minutes.

11. Pour off the chloroform and replace by benzene for 1 hour.

12. Embed in 3 changes of paraffin wax for 1 hour each.

13. Block edgeways, as the marrow is mainly on one side of the disc, and cut sections at 3 μ.

It is important to cut the sections with a slow action to avoid crumpling.

Staining of the sections may be carried out by any suitable method. Good results have been obtained with Weigert's iron hæmatoxylin and eosin-yellowish, Jenner-Giemsa stain for sections, Delafield's hæmatoxylin and azur-giemsa, Hynes's modification of Leishman's stain and by methods for reticulin impregnation and inorganic iron (Perl's reaction).

Sections containing marrow tissue surrounded by blood clot are obtained. The preservation of the marrow architecture is good, and sufficient material is obtained to study the leuco-erythropoietic elements and their relationship to the fat spaces and capillaries. In conditions with hyperplastic marrows such as pernicious anæmia and the leukæmias, particularly extensive sections are obtained. As sternal puncture can be performed repeatedly, changes in the marrow histology can be followed.

The methyl alcohol-formol fixative has been found to be rapid and effective and little shrinkage of tissue occurs. The use of other fixatives, including Zenker's, is being investigated in an attempt to obtain improved preservation of the red cell series.

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THE SYNDROME OF LEUKANÆMIA:
REPORT OF A CASE

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(PLATES XV-XVII)

FEW cases of leukanæmia have been recorded in the literature. Of these only a minority conform to von Leube's original syndrome in which features of pernicious anæmia and myeloid leukæmia co-existed without the full picture of either being present (von Leube, 1900).

Many of the reported cases give such incomplete data that no exact conclusions can be drawn as to the true nature of the blood condition : some, for example, refer to a pernicious anæmia picture but do not give so much as a red cell count. In some cases (Kimura *et al.*, 1940), although an erythroid reaction has been present, it has in no way resembled that of pernicious anæmia. The nature of reported cases is further confused by the tendency of some authors to report a pernicious anæmia-like picture merely because of a high colour index. In many cases the evidence for true megaloblastic pernicious-like anæmia (as distinct from a macrocytic anæmia) is scanty. Frequently it is not clear whether authors are referring to megaloblasts of Ehrlich (whose presence in the bone marrow we consider essential for this diagnosis) or the megaloblasts of American authors (Sabin, 1921 ; Doan *et al.*, 1925), which are normal precursors of erythroid cells in the marrow and correspond to the pro-erythroblasts of British authors (Israëls, 1939).

Davis and Fitz-Hugh (1939) have described the occurrence of macrocytosis in 3 of 20 cases of straightforward leukæmia. No question of pernicious anæmia arose and these cases, as the authors

point out, were not cases of leukanæmia but of leukæmia in which macrocytosis was present. Forkner (1938) states that in leukæmia the anæmia is usually óf the normocytic type. Rich and Schiff (1936-37) have described a ·case in which two independent diseases, pernicious anæmia and chronic lymphatic leukæmia, co-existed. Sinek and Kohn (1930) describe the co-existence of pernicious anæmia and chronic myeloid leukæmia. Seemann and Krasnopolski (1926) have used the term leukanæmia to describe cases showing a leuco-erythroblastic reaction. None of these conforms to the sense in which von Leube used the term.

Finally there are the cases which correspond to von Leube's original description by simultaneously presenting features of leukæmia-like and of pernicious anæmia-like (megaloblastic) blood and bone marrow. Of the 31 cases we have found in the literature, including von Leube's original case, only 9 appear to us, from the data available, to be acceptable as leukanæmia in the original meaning of the term. These are the cases described by von Leube (1900), Luce (1903), Parkes Weber (1904), Mattirolo (1905), Drysdale (1907-08), Treadgold (1913), Symmers (1921), and the 2 cases of Siebke (1927). Even in these cases a description of the " megaloblasts " which were present is not given and it is difficult to know whether to accept them as megaloblasts of Ehrlich or not. As all except two of the papers precede those of Sabin and of Doan *et al.*, who use the term megaloblast for the precursors of normal erythroid cells, we presume that it is to the megaloblasts of Ehrlich that the authors refer. The cells for which we reserve the term are the typical Ehrlich's megaloblasts with very fine network, open nuclei and smooth hæmoglobinised cytoplasm (Turnbull, 1936 ; Schulten, 1937 ; Israëls, 1939).

We are in agreement with Drysdale that leukanæmia, as a disease *sui generis*, does not exist, the cases which conform to von Leube's description being either atypical pernicious anæmia or atypical myeloid (usually myeloblastic) leukæmia. At the same time we think the term a useful one by which, in the original sense of von Leube's definition, to indicate a hæmatological condition in which there co-exist a pernicious anæmia-like and a leukæmia-like blood picture, with corresponding marrow disorder of the erythroid and myeloid tissues.

We present the following case record because it corresponds to the original description of the syndrome and because it raises certain questions relating to the maturation of the erythroid and myeloid cells.

CASE REPORT

Clinical history

Mrs. O. E. C. Female, aet. 24 years.
Previous history. Scarlet fever and measles in childhood. " Abscesses " in both ears at 7. tonsillectomy at 14 and appendicectomy at 18. Laryngitis 18 months previously. No history of drug medication, sedatives, headache

PLATE XV

LEUKANÆMIA

FIG. 1—Bone marrow showing normoblastic erythropoiesis   Wright's stain
× 1000

FIG. 2—Bone marrow showing megaloblastic erythropoiesis   Wright's stain   × 1000

powders (other than aspirin), radiation therapy or benzene contact was elicited.

The patient, a primipara in the fifth month of gestation, was admitted to a nursing home on 17.8.42 with generalised œdema. She also complained of acute abdominal pain, vomiting and severe headaches. " Spots in front of the eyes " were followed 3 days later by blindness in the left eye due to retinal hæmorrhage. There was bilateral papilloedema. Blood pressure was 180/105. The urine was heavily loaded with albumin. The case was diagnosed as toxæmia of pregnancy and abortion was induced by rupturing the membranes on 18.8.42. The child was born alive but died 7 hours later. There was very little blood loss. The only hæmatological investigations carried out at this time were: hæmoglobin 37 per cent. (5·2 g. per 100 c.c.), erythrocytes 1,890,000 per c.mm. (20.8.42). Anahæmin (1 c.c.) was given daily. The only other therapy was luminal grain 1 *nocte*. The table shows the blood count on 3.9.42.

TABLE

Hæmatological findings

	1942					1943					
	3/9	28/9	20/9*	1/10	6/10	29/4*	29/4	7/6	7/6*	15/6	5/7
Hæmoglobin (g. per cent)	4 3	6 3		5 3	8 2		4 5				3 64
Erythrocytes (millions)	1 47	2 34		1 47	2 87	28 4	1·11	3 43	123 6	41	70
Leucocytes (thousands)	6 0	4 2	58 1	2 7	1 6	4 2	3 3	30	83 5	81·5	80 5
Myeloblasts (per cent)			2 2	1		0 4	30	0 5	80	5	5
Pro-myelocytes (per cent)	7	1	0 8	1				6 5	2 8	3 5	4
E. myelocytes (per cent)	2	3	7 6	1		0 4	2		6 0	1 5	
N. metamyelocytes (per cent)	1		0 2				2		0 2		
			4 2	1	1			0 5	1 0	0 5	0 5
E. metamyelocytes (per cent)			0 4		1						
Stab cells (per cent)	10	18	6 0	15	23	1 6	27	4	1 4	4	4
Segmented neutrophils (per cent)	37	44	0 8	32	32	0 8	3	1	0 2	1	0 5
Eosinophils (per cent)			0 2		4						
L. mononuclears (per cent)	1		2 0	2	1	0 6			1 8	6	5 5
Lymphocytes (per cent)				44	38	36	22	10	3		
						42 4			1 4	0 5	
				40/100	40/100	19 0	63/100		Very		
						25·2		{	scanty		
Laluen (per cent)			Scanty				6/100				
Unidentified (per cent)			1 6	3		1·8	5				
Megalokaryocytes (per c. mm.)			6			1 8			1 6		Auer bodies in myeloblasts
M.C.V.											
Myeloid/erythroid ratio	29 2	27	1·3 2	36	28 5		46 5		21.1		

* Bone marrow (" Leucocytes " = total nucleated cells)

i Reticulocytes on 29 0 42 and 1 10 42 were 14 per cent Between 4 5 43 and 29 5 43, during intensive liver therapy, they fluctuated between 1 and 6 5 per cent

ii On 21 6 43 parsmyeloblasts were observed in peripheral blood films

iii Platelets on 7.6 43 were 37,700 per c mm

iv M C V. on 29 9 42 was 100 c µ; on 29 4 43 110 c µ

v M C D (Price-Jones):
   29 9 42 7 5 µ; σ = 0 01 µ; Macro = 6 8 per cent ; V = 12 per cent.
   18 12 42 7 1 µ; σ = 0 46 µ; V = 6 5 per cent.

vi Indirect positive Hijmans van den Bergh reaction was given on 29 9 42 and 18 12 42, when serum bilirubin was 1 8 and 2 6 mg per 100 c c respectively.

Twenty-seven days after admission the patient left the nursing home fairly well, though still dyspnoeic and confined to bed most of the time. On 17.9.42 she re-entered the nursing home because of " acute anæmia ". A blood count on 28.9.42 showed no appreciable improvement in hæmoglobin or red cells (table). A few immature myeloid cells were again found in the blood smear. The clinical response to intensified liver therapy being unsatisfactory bone marrow (fig. 1) was submitted to us for examination (29.9.42), with the results shown in the table. The peripheral blood on the same day showed 5·6 g. per

100 c.c. hæmoglobin and 1·47 million erythrocytes per c.mm. Total leucocytes were 6000 per c.mm.

When the case first came under our observation (1.10.42), the patient had already received liver therapy. As a result of this the megaloblastic nature of the erythroid reaction in the marrow received on 29.9.42 was not clearly apparent (fig. 1). After a long search 1 or 2 rather doubtful megaloblasts of Ehrlich and scanty giant stab cells were observed. On the basis of these cells and the macrocytic anæmia, we regarded the case as one of megaloblastic macrocytic hyperchromic anæmia in which the typical picture had been obscured by liver treatment. It has been our experience that giant stab cells persist in the marrow of cases of pernicious anæmia after liver therapy is instituted for a longer period than megaloblasts. We have found them useful in making a diagnosis of megaloblastic anæmia in retrospect when, in other respects, therapy had obscured the hæmatological picture. The longest period after which we have observed giant stab cells in the marrow after commencing therapy in a definite case of pernicious anæmia is 12 days. In this case our assumption, based on these cells, seemed to be confirmed when the patient's blood picture improved steadily with liver therapy. Unfortunately reticulocytes were not followed on this occasion but on 29.9.42 and 1.10.42 were 14 per cent. Treatment consisted of a blood transfusion (1500 c.c.) and liver therapy (Anahæmin and Procythol Forte). The table shows the blood on 6.10.42. Fragility of the erythrocytes in hypotonic saline was normal.

The patient was discharged on 14.10.42. She remained fairly well and was seen by one of us (H. F.) on 18.12.42. Her blood contained 9·9 g. per 100 c.c. of hæmoglobin and 3,530,000 erythrocytes per c.mm.

From this date until February 1943 she remained well and had no liver therapy. At the end of February, while on holiday, she developed tick-bite fever, with high temperature and body rash, and returned to Johannesburg in March acutely ill. She was admitted to a nursing home on 7.3.43. An immediate transfusion of 2000 c.c. of whole blood was given without reaction. As she improved greatly after the transfusion, no blood examination was carried out and she was not seen by us. Subsequently her condition slowly but steadily deteriorated. On 28.4.43 she was admitted to hospital complaining of severe palpitation, vomiting, headaches, weakness, dyspnœa, fatigability, loss of weight (126 lb. as compared with 168 lb. in January 1942) and profuse night sweats.

On examination the patient was noted to be pale, with a yellow tint of skin. She was dyspnœic and obviously very ill. The state of nutrition was good. There was slight clubbing of the finger tips, puffiness of the face (especially under the eyes) and some œdema of the ankles. Mucosæ and conjunctivæ were very pale but no ulceration of the mucous membranes was found. The teeth were in fairly good condition and there was no sponginess or bleeding of the gums, which were firm and healthy. The tongue was moist and furred. There was no glossitis. Apart from a precordial systolic murmur nothing of note was found on examination of the cardio-vascular system. Blood pressure was 136/90. The lungs were normal on percussion and auscultation. Nothing abnormal was detected in the central nervous system. There were no palpable lymph nodes. No petechiæ were found in the skin but one or two ecchymoses were present on the legs. There was definite sternal tenderness. The spleen, which was firm but not tender, was palpable 3 fingers' breadth below the costal margin. The liver was not enlarged. The ophthalmologist reported that the left eye was blind, while the right showed macular hæmorrhage apparently undergoing absorption, and one other small round retinal hæmorrhage.

Laboratory tests on 29.4.43 showed 4·5 g. per 100 c.c. hæmoglobin in the peripheral blood, with 1·11 million erythrocytes and 3300 leucocytes per c.mm. Thirty-nine per cent. of the leucocytes were myeloblasts ; 2 per cent. neutrophil
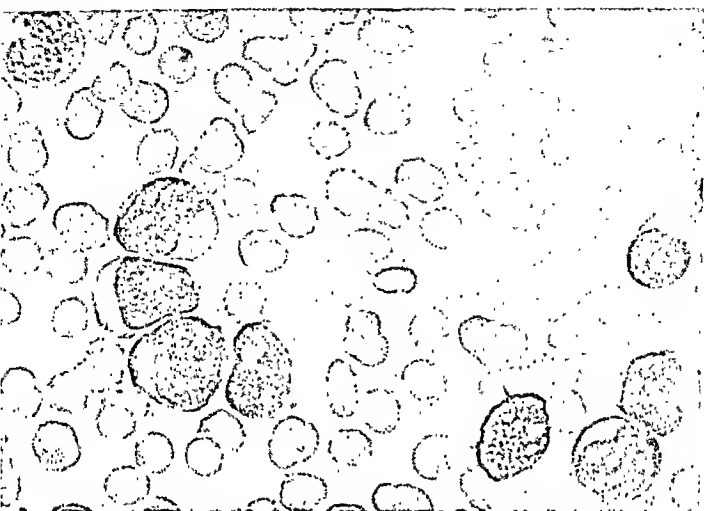
LEUKANÆMIA

FIG. 3.—Peripheral blood showing numerous myeloblasts. Total leucocyte count 30,000, with 83·5 per cent. myeloblasts. Wright's stain. × 1000.

FIG. 4.—Bone marrow showing myeloblastic leucopoiesis. Wright's stain. × 1000.

myelocytes; 2 per cent. neutrophil metamyelocytes; 27 per cent. stab cells; 3 per cent. polymorphonuclears and 22 per cent. lymphocytes. In addition, 63 erythroblasts and normoblasts and 6 megaloblasts of Ehrlich were observed per 100 leucocytes. Five per cent. of the nucleated cells in the peripheral blood were unidentified. The M.C.H. was 40·5 γγ and the M.C.V. 110 c.μ. The differential count of the bone marrow, which contained 28,400 nucleated cells per c.mm., is shown in the table.

The bone marrow now showed an even more marked erythroid reaction than it had done 5 months previously and the reaction was definitely megaloblastic in type (fig. 2). The myeloblasts in the marrow were 4·2 per cent. although the blood showed 39 per cent. In view of the definite macrocytic hyperchromic anæmia with numerous megaloblasts of Ehrlich in the blood and marrow, we interpreted the immature granular cells as a myeloid reaction in pernicious anæmia. The Hijmans van den Bergh reaction (2·0 mg. per 100 c.c.), the strongly positive Schuum test, the high M.C.V. (110 μ³), M.C.H. (40 γγ), M.C.H.C. (35·2 per cent.) and C.I. (1·4), and the abnormal myeloid/erythroid ratio with erythroid predominance in the marrow, seemed to confirm this view.

As the patient was too ill for a gastric analysis, liver therapy was immediately instituted. The reticulocytes were counted daily by the wet technique. Over a period of 24 days they did not exceed 6·5 per cent. in spite of adequate therapy with 3 different brands of liver extract (Anahæmin, Reticulogen and Examen). Four weeks later (3.6.43), tender enlargement of the cervical glands appeared. The temperature began to swing and for the remainder of her illness remained between 100° and 104° F., except for an occasional brief fall to normal. A feature of the case was the frequent heavy sweats which became more marked as the disease progressed.

Because of the failure to respond to liver therapy the diagnosis was reviewed (3.6.43). The blood leucocytes had now increased to 23,000 from the previous leucopenia. The persistently enlarged spleen and the high temperature cast further doubt on the diagnosis of pernicious anæmia. No focus of infection could be found. A gastric analysis showed free acid (= 23 c.c. N/10 NaOH) 30 minutes after histamine, thus ruling out true pernicious anæmia. The resting juice showed no free acid. The whole of the sternum was now exquisitely tender, suggesting leukæmia. We have never noted this sign in pernicious anæmia but find it in a high proportion of cases of leukæmia. On 4.6.43 small glands became palpable in both axillæ, tending to confirm the revised opinion that we were dealing with a case of atypical leukæmia. Another sternal puncture was therefore carried out (7.6.43). It showed a complete change of picture (fig. 4). The bone marrow now contained 123,800 nucleated cells per c.mm. of which 80 per cent. were myeloblasts and the M/E. ratio was 21 : 1. Megaloblasts of Ehrlich were present but very scanty. Indeed all erythroid cells had almost completely disappeared, to be replaced by 80 per cent. of myeloblasts. The latter cells were peroxidase-negative. Their characteristics are described under the section on histology. The peripheral blood on the same day showed 30,000 leucocytes per c.mm., of which 83·5 per cent. were myeloblasts (table and fig. 3).

On these findings the case was diagnosed as one of acute myeloblastic leukæmia with atypical onset and course. From this time on the peripheral blood showed 30,000 to a terminal 189,000 leucocytes per c.mm. Myeloblasts always formed 80·90 per cent. of the total. Paramyeloblasts, which Schulten regards as pathognomonic of myeloblastic leukæmia, were observed on 21.6.43 and 24.6.43. Auer bodies were present in the cytoplasm of the myeloblasts on 15.6.43.

On 16.6.43 the glands on the left side of the neck became exquisitely tender and considerably enlarged. A week later there was induration, swelling and

erythema of the left side of the face and neck suggesting abscess formation. On 6.7.43 the patient collapsed, became stuperose and developed persistent hiccough. Death occurred on 18.7.43.

Autopsy

Post-mortem examination was carried out by Dr B. J. P. Becker 24 hours after death. The significant findings were as follows.

Heart. Acute bacterial endocarditis involving the aortic valves.

Spleen much enlarged (1430 g.). It showed a fibrinous exudate on the surface. On section it was a dull reddish colour and showed obliteration of the Malpighian follicles. Multiple pale infarcts were present and some were septic.

Liver much enlarged (2275 g.) and showed fatty change.

Bone marrow in the sternum, ribs and femur was soft and of a greyish red tinge. In the femur active marrow had extended throughout the length of the shaft, causing thinning of the cortical bone.

Lymph nodes throughout the body were enlarged, soft and of a pinkish grey colour. The glands in the left cervical region were septic and had formed a large abscess.

Histology

The marrow in the femur and rib (figs. 5 and 6) is grossly hypercellular and shows very active mitosis. The increased cells are mainly large mononuclear cells similar to those noted in the vessels and in the lymphoid and splenic tissue. Some eosinophil myelocytes are present but erythroid tissue is inconspicuous. There are numerous megakaryocytes.

The liver shows infiltration by large mononuclear cells which are found mainly in the sinusoids. The splenic pulp is packed by similar cells but the follicles (which are inconspicuous) show no infiltration. The structure of the lymph glands is destroyed and their substance occupied by the abnormal cells. In the kidney the mononuclear cells are found only in the lumen of the vessels and not in the interstitial tissue.

The large mononuclear cells which are present in large numbers in the bone marrow (fig. 6), spleen and lymph nodes and in the vessels of all the organs examined, show the following characteristics in paraffin sections. They have central nuclei occupying 4/5ths of the cell. With Wright's stain, the nuclear chromatin is reticular, with nodal thickenings and rather inconspicuous nucleoli. The nuclear membrane is well defined. The nucleus is generally round but not infrequently indented, irregular and sometimes folded. The cytoplasm is smooth, agranular and weakly eosinophilic. In smear preparations of marrow taken during life and stained by Wright's method (fig. 4), the blast cells are 17 or 18 μ in diameter. The cell border is well defined and shows no pseudopodia-like structures except where there is distortion of the cell. The basophilic cytoplasm stains most deeply at the periphery and shows an area of perinuclear pallor. The most immature cells are agranular, but azurophil granulation is present in the majority. The granules increase in number and size as the cell

LEUKANÆMIA

Fig. 5.—Section of bone marrow (rib) showing hypercellularity due to leucopoietic proliferation   H. and E   ×95.
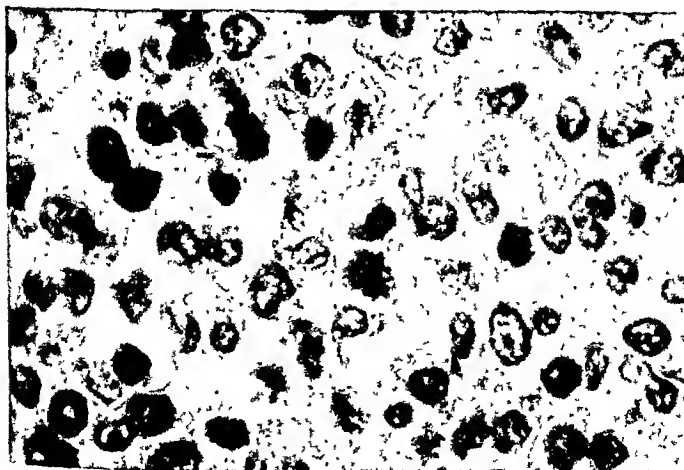

Fig. 6.—Section of bone marrow (rib) showing predominance of myeloblasts in the leucopoietic proliferation.   H. and E.   ×1230.

matures towards the pro-myelocyte, which contains numerous granules. Occasional Auer bodies are present. The nucleus, occupying 4/5ths of the cell, is, as in paraffin sections, round or oval and occasionally indented, but shows no folding. Where there is no compression by neighbouring cells the nucleus is central. The chromatin is fine and leptiform and no nodal thickening has been observed. Nucleoli are usually two in number, occasionally single, but are frequently more numerous, with a maximum of 6. They are clearly defined but seldom show paranucleolar condensation of the nuclear chromatin. The nucleoli are oval and where several occur in one cell are unequal in size. All gradations of cell maturity are seen from the myeloblast through the pro-myelocyte to the mature cells of the myeloid series. The myeloblasts are peroxidase-negative.

DISCUSSION

The only case we have found in the literature almost identical with ours is that reported by Treadgold. In it there was the same clinical course, with a leucoponia at the onset and terminal leucocytosis with a high myeloblast count in the peripheral blood. The autopsy and histological findings were also similar.

The possibility of the coincidental existence of pernicious anæmia and of leukæmia does not arise in our case as the presence of free acid in the gastric juice ruled out pernicious anæmia (*cf.* Wintrobe, 1942). Nevertheless there was abnormal maturation of the erythroid cells as evidenced by the presence of megaloblasts of Ehrlich. It seems obvious, in retrospect, to accept the case as one of leukæmia from the onset, but it is difficult to reconcile this with the abnormal myeloid/erythroid ratio with an excess of erythroid cells on 29.9.42 and 29.4.43 and the numerous megaloblasts of Ehrlich in the bone marrow. As pernicious anæmia did not exist in this case and the question of coincidence therefore does not arise, there existed a disease entity distinguished by macrocytic hyperchromic anæmia (with megaloblasts of Ehrlich in the marrow) and myeloblastic leukæmia. The possibility of the whole syndrome being due to pathological maturation (? deficiency of some unknown hæmopoietic factor or factors) will be discussed later.

From the reports of the 9 cases which we regard as probably leukanæmia, some points deserve emphasis. As a rule it is a manifestation of atypical leukæmia—one which is usually acute and of the myeloblastic type. The leucocyte count in the peripheral blood varies greatly from case to case and at different times in the same case (Treadgold). There is a macrocytic hyperchromic anæmia which has as its background a megaloblastic change in the bone marrow. Terminally the marrow may show a predominantly myeloid or a combined myeloid and megaloblastic change.

Clinically the cases are acute. The patients are pale, with a

yellowish tint of the skin and there is slight generalised œdema, particularly under the eyes. Retinal hæmorrhages are present. There is splenomegaly and the lymph glands are enlarged. Tenderness of the bones is often a prominent sign. Gastric achlorhydria may or may not be present. It is clear from our case that liver therapy can lead to masking of the megaloblastic reaction in the bone marrow, but does not necessarily cause the reticulocytosis seen in cases of pernicious anæmia.

At autopsy the findings are those of leukæmia with, in cases which have not been treated with liver, a megaloblastic change in the marrow. Absence of siderosis in the liver has been emphasised by some authors (Symmers ; von Leube ; Drysdale) ; others (Treadgold ; Siebke) have shown its presence, as in our own case, where it might have been due to the numerous transfusions. No such explanation can apply to the cases of Treadgold and Siebke.

A particular point of interest raised by this case is the presence of megaloblasts, giant stab cells and increased myeloblasts in the same marrow. It has been suggested that the red cell changes in leukanæmia are due to a disturbance of erythropoiesis by the proliferating myeloid tissue (Whitby and Britton, 1942). In our case, however, the erythroid disturbance existed at a time when myeloid proliferation in the marrow was inconspicuous. Megaloblasts of Ehrlich are known to occur as the result of defective maturation of the erythroid series. Dameshek and Valentine (1937) suggest that whatever deficiency is responsible for the abnormal maturation of the red cells also affects white cell maturation, with production of bizarre forms of " metamyelocytes " (giant stab cells). These, as we have pointed out (Foy and Kondi, 1943), are just as characteristic of macrocytic anæmia as are the megaloblasts of Ehrlich.

In pernicious anæmia, the absence of a known hæmopoietic maturation factor leads to defective maturation of erythroid cells and aberrant maturation of myeloid cells. In agranulocytosis, whose pathogenesis appears to be a maturation arrest of the myeloid cells, the predominant cells in the bone marrow are the pro-myelocytes and myeloblasts (Wintrobe). In the case recorded above all these forms of aberrant cell maturation were present. Megaloblasts of Ehrlich, giant stab cells and numerous myeloblasts occurred simultaneously in the marrow throughout the disease, though at one time the megaloblasts predominated and at another the myeloblasts. Jones (1943) has suggested that megaloblasts may be formed from myeloblasts (lymphoidocytes). Whether the megaloblasts in this case developed from myeloblasts or whether the latter were formed in excess and the former were present each as the result of abnormal maturation from a common stem cell is not clear. The failure of the marrow to return entirely to the normoblastic form of erythroid maturation with adequate liver therapy followed by an increasing proportion of myeloblasts suggests the possibility of a maturation defect in the

stem cell common to the erythroid and myeloid series. The possibility of leukæmia being a deficiency disease has been considered by Castle *et al.* (1934–35), but they met with negative results in attempting to treat chronic myelogenous leukæmia as such. A recent case gave us the opportunity to test the same theory in acute aleukæmic myeloblastic leukæmia. The results were negative. Massive doses of liver orally and parenterally, vitamin C, vitamin B complex, iron adenylate and pentose nucleotide had no effect on the blood picture. Over a period of weeks the patient received orally the ground-up tissues of chick embryo in the fresh state. This also had no effect on the blood or marrow picture. At autopsy the findings were those of myeloblastic leukæmia limited to the bone marrow. There was myeloid hyperplasia of the marrow with a high proportion of myeloblasts but no evidence in any other organ of myeloid infiltration or metaplasia. This suggests that though, at present, such cases must be classified hæmatologically with the leukæmias, it is possible that some of them are due to a maturation defect in the myeloid tissue of the marrow. Failure to substantiate the theory by treating one or two chronic and acute cases with known hæmopoietic stimulants neither vitiates nor disproves the theory.

The case reported in this communication seems to exhibit marked abnormalities of maturation affecting myeloid and erythroid cells simultaneously. The case showed leukæmic changes in the viscera at autopsy and was from that point of view a case of myeloblastic leukæmia. But the association of myeloblasts, the majority of which failed to mature beyond that stage, with megaloblasts and giant stab cells, raises the question whether leukanæmia may not be a manifestation of an extensive maturation abnormality in the hæmopoietic tissue in which the erythroid and myeloid cells are each affected by the absence of some unknown factors. Alternatively it might be postulated that defective maturation results from the absence of some factor or factors necessary for the normal maturation of their common stem cell, the hæmocytoblast.

SUMMARY

1. A case of leukanæmia is described, with complete hæmatological, clinical and post-mortem findings. It commenced as a typical macrocytic anæmia, with Ehrlich's megaloblasts and giant stab cells in the sternal marrow, the latter being regarded as just as characteristic of the macrocytic anæmias as are the megaloblasts of Ehrlich. Free acid was present in the gastric juice after histamine.

2. In the later stages of the disease the blood became leukæmic, with 30,000–189,000 white cells per c.mm., 80–90 per cent. of which were myeloblasts; some paramyeloblasts were present which are regarded as pathognomonic of the myeloblastic leukæmias.

3. It is suggested that the red and white cell changes which occur

in leukanæmia are due to a maturation defect in the stem cell (hæmocytoblast), which affects both the erythroid and myeloid series, and that the defect may be due to the absence of some unknown factor or factors necessary for the proper maturation of the hæmocytoblast and the orderly formation and development of both red and white cells.

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THE RELATIVE SUSCEPTIBILITY TO PHAGOCYTOSIS OF *GRAVIS* AND *MITIS* TYPES OF *C. DIPHTHERIÆ*

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Dr Jean Orr-Ewing died on 17th November 1944: an obituary notice appeared on p. 149 of the last issue of the *Journal*. The present paper was prepared by J. W. McLeod from the rough draft of a communication made by the author at the meeting of the Society in January 1938. Unfortunately some of the figures on which the tables and text depend were not available and in consequence there was difficulty in elucidating certain minor discrepancies between table I and the text. It was obvious, however, that a very considerable body of careful work had been carried out which might well throw light on some of the outstanding problems with regard to the relative pathogenicity of *mitis* and *gravis* strains of *C. diphtheriæ*. The observations, if corroborated, open up a new and interesting line of attack on these problems. The original text has therefore been slightly adjusted to bring it into line with the tables and a footnote to the tables explains the interpretation that has been adopted. In addition, a correction of the statistical argument has been introduced on p. 171 where the correlation of the two variables is discussed. The references to the literature have been brought up to date.

SINCE the *gravis, mitis* and *intermedius* types of *C. diphtheriæ* were first described (Anderson *et al.*, 1931, 1933) it has been generally agreed that the nomenclature has been justified by their clinical behaviour. It is proposed in this paper to consider only the two more divergent types, *gravis* and *mitis*. Briefly, *gravis* has been shown to cause a higher case mortality rate—roughly four times that caused by *mitis* (McLeod, 1943)—and a higher incidence of infection in Schick-negative individuals (Robinson and Marshall, 1935 ; Robertson, 1943 ; Grant, 1945). There is also some evidence that *gravis* organisms are not only more invasive than *mitis* (McLeod, Orr and Woodcock, 1939) but also more resistant to phagocytosis (Ørskov *et al.*, 1944). Many *gravis* infections are typically toxic in character. In short, the average *gravis* organism is more toxic and probably more invasive than *mitis* in the human body. The mechanism of this difference in virulence is not known. It has been suggested that *gravis* toxin differs qualitatively from other diphtheria toxins (Etris, 1934 ; Clauberg, 1939) ; but Parish, Whatley and O'Brien (1932 *a* and *b*), Frobisher (1943) and Zinnemann (1943, 1946) were unable to obtain evidence of this. Again O'Meara (1940) has advanced evidence for a secondary toxic factor distinct from the classical toxin, and while

the work of Robertson (1943) suggests that this may be possible, McClean (1941) and Frobisher and Mauss (1943) were unable to find such substances in any significant concentration.

The situation may therefore be summed up by saying that there is at present no generally accepted proof that *gravis* strains produce unusual toxic elements, although this possibility has not been finally excluded. The facts may be explained on the hypothesis that the *gravis* type produces ordinary diphtheria toxin in larger quantities than *mitis*, a hypothesis strengthened by Mueller's (1941) demonstration that *gravis* strains are best adapted for producing toxin in the presence of excess of iron in the medium and Zinnemann's (1943) extension of this observation to a larger series of strains, using a medium rich in iron and corresponding more nearly to the composition of animal tissues than that used by Mueller. The observations of Murray (1935) and of Gundel and König (1938) showing inferiority of standard antitoxin and prophylactic in protecting against *gravis* strains could also be explained by this hypothesis. There are two possible ways in which this might arise : (i) by the production of more toxin per unit organism, (ii) by more rapid multiplication of the bacteria in the tissues owing to a higher resistance to the various defence mechanisms of the body, *i.e.* a higher survival value. The two mechanisms might, of course, act in combination. Ørskov *et al.* have attempted to test the first possibility.

The investigations now described are, however, directed to determining, by observations on rates of multiplication under conditions approximating to those obtaining *in vivo*, the relative resistance of *gravis* and *mitis* strains to the bactericidal action of whole blood.

Methods

A modification of the blood bactericidal method originally described by Todd (1927) was adopted. In this method 0·5 c.c. of defibrinated blood is placed in each of a number of small test-tubes and descending dilutions of the organism under test are added in series. The tubes are then sealed and incubated at 37° C. in a rotating apparatus which ensures thorough mixing of the contents without frothing. At the end of the period of incubation (usually 24 hours) the tubes are opened and samples are plated out. In the original method the bactericidal power of the blood towards a given organism is expressed by the number of bacteria killed, and this number is calculated from the inoculum to the last tube in the series found to be sterile on subculture.

This method has very great advantages and is undoubtedly the method of choice for the investigation of large differences in resistance such as occur for instance between rough and smooth strains of a given organism. The method as it stands, however, is not a delicate one and there appear to be great technical difficulties involved in adapting it to the detection of the relatively small differences in resistance which may be expected between two closely related pathogenic organisms. Moreover, when destruction depends upon phagocytic action (as in the case of diphtheria bacilli) the method demands that one of the organisms under test shall be rather highly susceptible to phagocytosis, since otherwise the sterilisation of reasonably large numbers does not take place within the rather limited time (3-6 hours) during which the phagocytes are

fully active. It was found that phagocytosis of diphtheria bacilli was rather slow. Finally the method does not measure bactericidal action as such, but the final result of killing versus multiplication of the organisms.

For these reasons I have abandoned the criterion of complete sterilisation and have further modified the test by the inclusion of a second series of tubes each containing 0·5 c.c. of blood from which the leucocytes have been removed by filtration through cotton wool in accordance with the method recommended by Fleming (1926). Such blood is completely stripped of bactericidal power towards *C. diphtheriæ*. In the method finally adopted, therefore, equal inocula of a suitable dilution of the bacterial suspension are added to each of a pair of tubes containing (*a*) normal defibrinated blood and (*b*) deleucocyted and defibrinated blood. In tube (*a*) multiplication and destruction take place on incubation, in tube (*b*) multiplication only. It is assumed that, other things apart, the rate of multiplication would be equal in the two tubes. A measure of phagocytosis in the system is therefore obtained by comparing the number of living bacteria in tubes (*a*) and (*b*) at the end of the period of incubation. A unit volume is plated out from each tube on a transparent solid medium (Douglas's trypsin serum agar) and colony counts are simplified by throwing the enlarged image of the plates on a screen and marking off colonies as they are counted. The relation is expressed as the percentage survival in normal blood thus :—

$$\frac{\text{No. of colonies per unit volume normal blood}}{\text{No. of colonies per unit volume deleucocyted blood}} \times 100.$$

The multiplication rate may be obtained by comparing colonies from deleucocyted blood before and after incubation.

Observations

Three dilutions of each strain were used. *Gravis* and *mitis* strains were always put up in pairs and every effort was made to treat each member of the pair in exactly the same way. In all, 26 pairs of strains were tested in this way against the blood of 16 subjects. The strains were obtained from eleven different districts and included the three principal British serological types of *gravis* and at least seven strains of *mitis* type. The subjects from whom the samples of blood were obtained were members of the University and laboratory assistants. They were found to fall into two groups (table I) :—group I (13 in all), whose blood failed to kill most *gravis* strains as readily as it did most *mitis* strains : group II (3 in all), whose blood killed *gravis* strains as easily as or more easily than *mitis* strains. Not all *gravis* strains were superior to *mitis* strains in their resistance to the bactericidal power of whole blood, however, and the results obtained with 26 pairs of strains and 16 samples of blood—including both groups—are compared in table I, in which the relative resistance of *gravis* and *mitis* strains is stated without detailed figures for percentage survival.

In the tests of group I blood samples with the 26 pairs of strains, results have been weighted in favour of *mitis* by considering any case in which one of several observations showed better survival of *mitis* as " ties " and those in which equal numbers of *gravis* and *mitis*

TABLE I—*Summary of results with ... n text with one or more pairs*

Date	Subject	Schick	Blood antitoxin (units per c.c.)	Gravis strains Strain	Type	Origin Place	Clinical	Mitis strains Strain	Type	Origin Place	Clinical	Total	Gr>M	M>Gr	Gr~M
				Group I (non-killer of gravis)											
2.11.31	J. O. L.	−(N)		L.S.D.S.	A	Leeds	Severe	L.42	a	London	Mild				
9.11.34	" "			S.111	B	Stafford	"	O.Ch.	a	Oxford	"				
26.11.34	" "			L.S.N.S.	A	Leeds	"	H.12	c	London	"				
9.12.31	" "			H.219	C	Hull	Fatal	H.11	c	Hull	"				
14.12.31	" "			New	A	Alton	Moderate	A.Tr.	c	Abingdon	Mild				
14.12.31	" "			Date	A	Abingdon	Mild	A.Fa.	c	Abingdon	Mild				
16.1.35	Mc. G.			C.Cr.	H	Cork	Carrier	M.Vl.	c	Manchester					
16.1.35	" "			C.Mc.	H	"	Caves in immunised subjects	M.I.	c						
12.3.35	D. R.	−(S)		W.977	B	Austria	Carrier	W.6171	c	? Manchester					
28.1.35	" "	−(S)	1:250	W.956	A	Swanley	"	M'	c	Manchester	?				
25.2.35	J. W.	−(A)	1:5	W.957	A	Liverpool	Moderate	W.642	c	Manchester					
10.3.37	" "			H.781	A	Hull	Fatal	H.53	c	Liverpool					
31.12.36	" "			H.519	A	Leeds		L.537	c	Leeds					
7.1.37	F. P.	−(A)	1:10	L.Man.	A	Hull		L.337	c	Leeds	Mild				
1.2.37	" "			H.518	C	London	"	L.337	c	London	"				
16.12.36	M. B.			L.C.C.1008	G	Cork	"	L.C.C.10970	c	Leeds	?				
26.1.37	M. P.			L.C.C.10993	A	Leeds	?	L.C.C.10970	c	Leeds	?				
25.1.37		+++++	<1:1000	C.St.	B	Cork	Mild	L.C.C.1053	c	Leeds	?				
2.2.37		+|+		L.583	A	Leeds	Moderate	L.Cr.	c	Leeds	Mild				
22.3.37			<1:1000	H.546	A	Hull	Severe	H.5691	c	Liverpool					
22.3.37	A. H.			H.172	A	Liverpool	Fatal	L.537	c	Leeds					
15.5.37	A. D. G.	−(A)		H.518	C	Hull	Fatal	A.In.	c	Abingdon	?				
7.11.36	H. E.	+|+	<1:1000	K.D.	A	Oxford	Mild	L.C.C.3326	c	London	Mild				
2.3.37				L.D.	A	London	?	L.170	c	Liverpool					
2.5.37	R. L. V.	+|+		H.580	A	Leeds	Moderate	H.429	c	London					
25.5.37		−(A)		L.C.C.0103	A	Liverpool	Severe	L.C.C.10970	c	Leeds	Mild				
9.12.36	M. J.	?		H.510	C	Hull	Fatal	L.Cr.	c	Leeds	Moderate				
15.1.37	K. L.	?		H.572	A	Hull	Mild	L.M.	c	London	Mild				
	"			H.510	C	Hull	Fatal	L.537	c						
							Totals					73	61	7	2
				Group II (gravis killers)											
10.12.36	J. C. B.	−(A)		L.D.572	B	Leeds	Moderate	L.470	a	Leeds	Mild				
21.2.37	D. C.	+++		L.M.	A	Sheffield	Mild	L.M.	a	Leeds	Moderate				
21.2.37	"			S.3090	A	Sheffield	Severe	S.2923	e	Sheffield	"				
6.7.37	D. Ch.			S.3090				S.2923		"	"				
6.7.37	"			C.4	B	Cork	Mild	S.2923		"	"				
							Totals					11	5	3	3

It will be noted that no blood in this table actually kills *gravis* more readily than *mitis* if the total of all observations is considered. It is assumed that the correlation with the text (p. 172) is obtained by placing in group II the bloods D. C. (which shows 2 *mitis*>*gravis* ties), D. Ch. (which shows 3 *mitis*>*gravis* ties). Of the remaining 13 bloods, which are assumed to form group I, J. C. B., Mc. G., C. Cr., F. P., H. E., R. L. V. and K. L.) show a minority of *mitis* "wins" or "ties". It is assumed that in column 3 (Schick is+) N = naturally negative, A = negative after prophylactic treatment.

" wins " were recorded as *mitis* " wins ". Even so, the observations
show :—

> *Gravis* resistance to phagocytosis > *mitis* with 20 strains
> *Mitis*   „   „   „   > *gravis* „   3   „
>   Resistance equal,   „   3   „

There is, therefore, a variation in the relative resistance of *gravis* and
mitis strains to phagocytosis, that of the former being on the average
considerably greater.

Stated quantitatively for 26 pairs of strains and a total of 79
observations the comparison is as follows :—

	Average percentage survival of diphtheria bacilli in normal blood	Difference
Gravis . . .	27·49	17·7 ± 2·72
Mitis . . .	9·78	

$$\frac{\text{Difference}}{\text{Standard error of difference}} \quad \frac{17\cdot7}{2\cdot72} = 6\cdot50$$

It was found that the average rate of *gravis* multiplication slightly
exceeded that of *mitis*, but there were considerable individual variations
and the difference was not significant. The above results are
independent of this factor.

Since the relatively high *gravis* resistance is not common to all
strains, the question of sampling errors in the selection of strains
must be considered. On the assumption that the strains were drawn
from a population in which *gravis* and *mitis* strains were in reality
equal in their resistance to phagocytosis, one would expect, in a
sufficiently long series of comparative tests, to obtain an approximately
equal number of *gravis* and *mitis* " wins " plus an unknown proportion
of " ties ". If *mitis* is heavily favoured by considering all the " ties "
as *mitis* " wins " and only taking into account *gravis* " wins " and
gravis " non wins ", the probability of each is 0·5 and the chance
of obtaining either result 20 times in a series of 26 trials is 1 : 145.
By applying a similar argument to the samples of blood, we find
that the chance of drawing 13/16 of one kind from a population in
which the distribution is actually equal is 1 : 58. The chance of one
or the other of these events occurring accidently is therefore
$\frac{58+145}{58\times145} = 1 : 41$. The experimental result, therefore, attains a
considerable level of conventional significance.

The reason for the difference in behaviour between the two groups
is not clear. Capacity for killing *gravis* is certainly not connected
with a high level of antitoxin in the blood. The last point is most

clearly shown by the following series of experiments in which the "*gravis* killer" was Schick+ and had a blood antitoxin content of

TABLE II

Contrast between bloods of group I (non-killers of gravis) and group II (gravis killers)

	Group I		Group II	
	Gravis	*Mitis*	*Gravis*	*Mitis*
Average percentage survivors in normal blood	33	12	5	14
Difference (*gravis-mitis*)	$+20\pm5.25$		-9 ± 2.26	
Difference / Standard error of difference	$\frac{20.9}{5.25}=3.98$		$\frac{9.0}{2.26}=3.98$	
No. of strains : 7.	No. of observations : group I, 24; group II, 23			

Tests were made with a number of bloods from either group against the same strains.

<0·001 units per c.c., whereas the group I subject, a weak "*gravis* killer" was Schick— (artificially) and had 0·1 unit of antitoxin per c.c. (titrations kindly made by Mr Glenny).

Table III shows percentage survivors in $\dfrac{\text{normal blood}}{\text{deleucocyted blood}}$.

TABLE III

Discrepancy between the antitoxic content of the blood and its gravis-killing activity

Group I. Blood (F.P.) Schick — : 0·1 unit antitoxin per c.c.				Group II. Blood (B.H.R.) Schick+: <0·001 unit antitoxin per c.c.			
Date	*Gravis* (L.C.C. 10,903)	*Mitis* (L.C.C. 10,070)	Difference (G.-M.)	Date	*Gravis* (L.C.C. 10,008)	*Mitis* (L.C.C. 10,070)	Difference G.-M.
16.12.36	40·0	2·8	+37·2	22.12.36	0·6	5·6	— 5·0
	10·0	0·5	+15·5		0·6	4·0	— 3·4
	34·0	3·3	+30·7		...	4·5	...
26.1.37	15·4	0·1	+15·3	13.1.37	0·9	11·7	—10·8
	27·4	0·3	+27·1		7·5	8·3	— 0·8
	13·6	0·4	+13·2		8·0	5·0	+ 3·0
Means	24·4	1·2	+23·2		3·5	6·9	— 3·4

Similar conclusions were obtained in other observations.

It was shown in the course of the investigation that the capacity for killing *gravis* may be acquired as the result of infection with a *gravis* strain. One of the group I subjects, whose blood had been repeatedly tested and found to have little capacity for killing any of the three main serological *gravis* types, contracted an infection with a serological type B strain. On testing his blood after the attack it was found to have acquired marked killing power for the serological types A and B, a complete reversal, whereas for *gravis* type C, and for *mitis* its killing power remained unchanged.

The *gravis*-killing power of the three unselected subjects in group II has not been explained; they had not had diphtheria. More work is required, however, to ascertain whether the carrier state is likely to induce this form of antibacterial immunity.

The paper is put forward in the hope that workers in *gravis*-infected areas may be stimulated to investigate (*a*) the effect of the carrier state on this manifestation of immunity, and (*b*) its relation to the antigenic differentiation of types of the diphtheria bacillus by the method of agglutination.

Summary

It is submitted that *gravis* type strains of diphtheria bacilli are on the average more resistant to phagocytosis when tested against the blood of most individuals than *mitis* type strains. It seems probable that this faculty accounts at least in part for their high clinical virulence.

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RELAPSING FEBRILE NODULAR INFLAMMATION OF ADIPOSE TISSUE (WEBER-CHRISTIAN SYNDROME): REPORT OF A CASE WITH AUTOPSY

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(PLATES XVIII-XXI)

THIRTY-ONE cases have now been reported of the syndrome which was first recognised by Weber (1925) and described by him under the title "Relapsing non-suppurative nodular panniculitis". To this designation Christian (1928) added "febrile".

The literature contains reports of only four fatal cases, three of which were examined *post mortem*. In the case reported by Tilden et al. (1940) the patient died from tuberculosis 8 months after the symptoms of panniculitis had subsided. Only the scar of a single previous subcutaneous nodule had been examined. Recently Spain and Foley (1944) reported a case of chronic glomerulonephritis which, during the week prior to death, presented evidence of the Weber-Christian syndrome. Small nodules were found, not only in the panniculus adiposus but also in the mesenteric, omental and pre-tracheal adipose tissue. Histological examination of the nodules revealed changes which were interpreted as showing the following sequence, namely, compact foci of foamy cells which later became necrotic and showed signs of inflammation. Another case with autopsy was reported in 1941 by Kritzler (quoted by Spain and Foley): the nodules presented similar appearances.

A fourth fatal case of the Weber-Christian syndrome with complete autopsy is described below

CASE REPORT

Clinical history. S. S., aged 37, house-wife, "was never ill" until June 1942. During the course of one month she developed a syndrome which was observed in three successive attacks. Death occurred about 9 months from the onset. The clinical course is summarised in table I and it is deemed sufficient to describe in particular the events of one attack—the first.*

For a fortnight the patient complained of general weakness, headache and fleeting pains in joints (knees, shoulders, fingers) but without fever. This was followed by an acute attack of fever of remittent type up to 39° and 40° C., accompanied by general malaise. Coincident with the rise of temperature there appeared nodules about the size of hazel-nuts in the subcutaneous tissues of

TABLE I

Summary of clinical findings

Date	Palpable nodules in panniculus adiposus (in chronological order)	Other symptoms, in retrospect attributable to lesions of adipose tissues	General symptoms
June 1942 . .			Weakness; inability to work
July 1-8 . fever 9-25 . „	1. "Hardening" in left calf 2. Right thigh 3. Below left breast		Headache; pain in joints
28 . August 1-15 „ .			Lytle fall of temperature Weakness; inability to work
Interval without symptoms (about three weeks)			
September 8 . fever 11 . „	4. Both thighs 5. Left arm	Retrosternal pain	Swelling of legs
October 1 . „	6. Right arm 7. Left groin (biopsy)	"Tumour" at the entrance of true pelvis	Diarrhœa; abdominal pain
16 . „ November . .		"Tumour" in pelvis decreased in size	Gradual decrease of temperature Inability to work; general weakness
Interval without symptoms till latter half of December 1942			
End of December 1942 January 1943			Chills; night sweats; no fever Gradual development of œdema in lower extremities
7 . fever	8. Several nodules in right groin 9. Right thigh (medially)		Diarrhœa; abdominal pain
10 . „ 15 . „ 18 . „ 23 . „	10. Left groin 11. Right hand (biopsy) 12. Right forearm 13. Left thigh (medial aspect)	Retrosternal pain	
28 . „		"Granular" thickening at entrance to true pelvis	Abdominal pain, left-sided
30 . „ February 1 . . .			Sudden fall of temperature Œdema of lower extremities extending on gluteal and sacral regions
4 . . .			Vomiting; dysphagia; peritoneal symptoms
February 8, 1943 . . Death			

the trunk and extremities. In the initial stage these were sensitive to pressure and the overlying skin was slightly cyanotic. After 2 or 3 days the nodules became firmer: they disappeared within 5-8 days. During the course of 3 to 4 weeks new nodules cropped up while the others gradually disappeared. During the whole of this period the fever remained of the remittent type, only decreasing gradually with the disappearance of the last of the nodules.

Laboratory findings. During the relapses a mild secondary anæmia was found and a moderate leucocytosis without abnormalities in the differential count. On one occasion only, a leucocytosis of 18,000 was present. A blood culture at this time gave no growth. Mantoux's tuberculin test was repeatedly negative. The Wassermann and Kahn testa were positive.

Neurological examination. Oppenheim's reflex was positive on the right side during both relapses. On several occasions patellar clonus could be obtained on the same side.

Biopsies from subcutaneous nodules were taken on two occasions : (*a*) from a firm nodule in the left inguinal region, taken during the first relapse ; histological diagnosis, lipogranuloma : (*b*) from a recent, fairly soft nodule on the flexor surface of the right forearm, taken during the second relapse ; histological diagnosis ; suppurative inflammation of adipose tissue.

Provisional clinical diagnosis. A primary disease of blood vessels was thought of, such as migrating thrombophlebitis or polyarteritis nodosa. In view of the repeatedly positive Wassermann and Kahn tests tertiary syphilis could not be excluded.

Treatment. During the first relapse medication with potassium iodide was twice attempted. On the first occasion the patient reacted with an abrupt rise of temperature and aggravation of malaise ; on the second, with a generalised skin rash and conjunctivitis.

Subsequent course. Four days before death there occurred an acute increase in the œdema of the lower extremities, which rose to the level of the vulva and gluteal and sacral regions. Simultaneously the abdomen became markedly distended. The temperature fell to 37° C.

Final diagnosis. Thrombosis of inferior vena cava. (Polyarteritis nodosa ? Tertiary syphilis ?)

Post-mortem examination

Anatomical diagnosis. Acute diffuse suppurative peritonitis due to *Strep. hæmolyticus* ; chronic tonsillitis, with small abscesses containing streptococci ; relapsing suppurative and granulomatous inflammation of adipose tissue throughout the body, predominantly in retroperitoneal space ; ecchymoses and ulcers of skin of lumbar, sacral and upper gluteal regions (histologically due to extension of suppurative panniculitis to arterioles, with subsequent thrombosis) ; relapsing parietal thrombosis of pelvic and iliac veins and of inferior vena cava below renal veins ; marked œdema of lower extremities, vulval and sacral regions ; paralytic distension of intestines ; " acute splenic tumour " ; acute ulcerative œsophagitis ; non-specific lingual ulcer ; healed small infarct of left kidney ; œdema of pia-arachnoid ; chronic prolapse of intervertebral disc (L. I/II) with slight compression of spinal cord ; so-called Schmorl's nodule in lower part of vertebral body (L. II) ; osteosclerosis of lower half of body of fifth vertebra.

Details of certain post-mortem findings

Autopsy was performed 2½ hours after death. The body was that of a short obese, middle-aged female. The abdomen was markedly distended and there was considerable œdema of the lower extremities up to the level of the iliac crests. In the lumbar and to a less extent the gluteal regions there were numerous ecchymoses and scattered circular ulcers with punched-out margins and smooth pale red floors 5–15 mm. in diameter.

Peritoneal cavity. The small intestine and colon were greatly distended by splashing fluid and gases. The peritoneal cavity contained about 1000 c.c. of turbid pale yellowish fluid, while the peritoneum itself was covered with yellowish grey friable exudate which could be swabbed away with ease. The intestinal loops were glued together by this exudate, especially in the left hypochondrium, where a collection of thick greenish grey pus was found between omentum, spleen and splenic flexure of colon. There were no firm adhesions. The lower margin of the liver reached the right costal margin. Cultures from the peritoneal fluid as well as from the thick pus in the left hypochondrium revealed haemolytic streptococci.

Examination of the gastro-intestinal tract, kidneys and descending urinary tract, uterus and adnexa, liver, pancreas and spleen failed to reveal inflammatory changes : infection of the peritoneum due to direct extension could apparently be excluded. Brain, meninges and middle ears were without gross morbid changes.

Adipose tissue. (a) *Panniculus adiposus.* The layer of fat in the anterior abdominal wall was 8·9 cm. thick, above the sternum about 3 cm. thick. The cut surfaces were bright yellow. The fatty tissue of the breasts was abundant and there were small areas of brownish grey firm tissue ; glandular tissue sparse ; on the right side a few small cysts. On the thighs the panniculus was about 8·9 cm. thick. The cut surfaces oozed abundant watery colourless fluid. There were some firmer areas, not clearly delimited and without difference of colour.

(b) *Retroperitoneal adipose tissue (including true pelvis, mesentery and omentum).* In these sites, fat was abundant and in many areas the cut surface presented innumerable greyish dots irregularly distributed. Discrete greyish foci were sometimes found 5·20 mm. apart ; elsewhere they were massed together into compact palpable firm nodules. These lesions were most fully developed in the pararectal, mesosigmoid, mesenteric and omental adipose tissue and around the kidneys and adrenals.

(c) *Intrathoracic adipose tissue.* This was abundant in the mediastinum and under the epicardium. In the anterior inferior mediastinum, close to the sternum, two softened nodules of adipose tissue were found which measured about 5 mm. in diameter and were orange-yellow in colour. In the subepicardial adipose tissue between the aortic root and left atrium there were a few tiny nodules, 1·2 mm. in diameter, of hard consistency and bright yellow colour.

Histological examination of organs

Tonsils. Focal suppurative inflammation within atrophic lymphoid tissue. One pus-distended crypt contained Gram-positive streptococci, singly and in clumps.

Liver. Slight fatty change in periphery of lobules. Focal dissociation of trabeculae and intrahepatic oedema. Periportal tissue contained many small round cells and scattered polymorphonuclear leucocytes, chiefly near small bile ducts.

Pancreas. Without pathological change, except for chronic inflammatory foci in the interstitial adipose tissue of the tail (*vide infra*).

Kidney. The scar of a small cortical infarct contained a few scattered leucocytes. The adipose tissue around the calices showed infiltration with neutrophil leucocytes and round cells with large pale nuclei. No other pathological change.

RELAPSING NODULAR PANNICULITIS

Fig 1—Biopsy from right forearm, 24 hours after a " swelling " was noticed   Acute inflammation infiltration with polymorphonuclear leucocytes   ×160

Fig 2.—From the same biopsy   Segment of a peripheral capillary showing stasis of leucocytes Emigration of the latter into intact adipose tissue   ×440

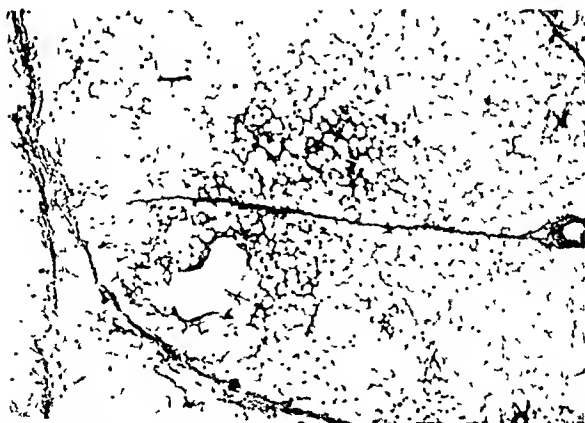Fig 3—Section from subcutaneous adipose tissue of right thigh which showed no gross lesion Early phase of acute inflammation exudation of leucocytes into centro lobular areas of the adipose tissue The terminal septum, which crosses between the foci of inflammation, is widened by accumulation of lymphocytes The neighbouring lobules show no infiltration   ×20

Sections from the *myocardium, genital organs, glands of internal secretion, diaphragm* and *ileo-psoas muscle* showed no pathological changes.

Histological examination of adipose tissue and related blood vessels and lymph nodes

Thirty-six blocks of adipose tissue from different regions were examined after fixation in formalin, Zenker's fluid, Benda's copper acetate mordant and Orth's fluid. Sections were stained with hæmatoxylin and eosin, hæmatoxylin and van Gieson, Weigert's elastic tissue stain, Gram's stain, Ziehl-Neelsen, cresyl violet, Petersen-azan and by silver impregnation (Foot). Fatty substances were stained with Sudan III in frozen sections and by Ciaccio's method.

The affected adipose tissue presented a variety of inflammatory lesions (table II). There were areas of (1) suppurative inflammation, (2) granuloma-like change, and (3) healed inflammation with residual changes.

1. *Areas presenting suppurative inflammation.* This type of lesion could be traced from the earliest stages of its development. For its study, biopsy material from a subcutaneous nodule which had been palpable for no longer than 24 hours was used, and autopsy material from the panniculus without palpable nodules.

In the recent biopsy nodule (right forearm), epidermis and dermis showed no change. The deep layer of subcutaneous adipose tissue showed abundant diffuse leucocytic infiltration (fig. 1) extending between the individual fat-cells which thus became progressively separated from one another, thereby loosing their polygonal and definite outline. Fat-cell nuclei had disappeared but the endothelial lining of capillaries remained recognisable. An isolated fragment of adipose tissue taken at some depth failed to show diffuse infiltration, but capillaries were prominent and contained blood in varying amount. Some of them were filled in limited stretches with abundant polymorphonuclear leucocytes (fig. 2) and there was evidence of diapedesis. The capillaries within the fibrous septa and the larger blood vessels showed no pathological change.

Adipose tissue from the right thigh also showed centro-lobular accumulations of polymorphonuclear leucocytes (fig. 3). The interlobular septa were thin, however, and unaffected, except where they traversed between areas of inflammation. There they were widened owing to accumulations of lymphocytes. The fat cells in the areas involved showed the same changes as those already described. In one infiltrated area there were several coalescing fat cells forming a cyst-like space. The capillaries in affected lobules were often engorged with polymorphonuclear leucocytes. The septal capillaries contained only scattered leucocytes in addition to erythrocytes. Arteries and veins were free from changes except slight lymphocytic infiltration of the adventitia of some of the smaller arteries.

2. *Granuloma-like lesions.* These consisted of accumulations of histiocytes, lymphocytes and foreign-body giant cells. They sometimes involved extensive areas of adipose tissue ; elsewhere there were

TABLE II

Histological findings in the adipose tissue of various regions of the body

A. Panniculus adiposus	
Acute stage	Right thigh (initial, pre-nodular lesion)
	Flexor surface of forearm (biopsy taken 3 weeks before death, from a 24-hour old nodule)
	Sacral and lumbar regions
Granuloma-like stage	Left inguinal region (biopsy taken 12 weeks before death, from a 5-day old nodule)
B. Retroperitoneal adipose tissue	
Acute stage *	True pelvis
	Mesocolon (relapse)*
	Adjacent to inferior vena cava
	Adjacent to cisterna chyli
Intermediate stage	Retroperitoneal adipose tissue (relapse) *
	Mesentery (root)
	Mesentery (middle)
	Parapancreatic adipose tissue
	Adjacent to parapancreatic lymph-node
	Greater omentum (free margin)
	Adjacent to renal hilum
Granuloma-like stage	Mesentery (root)
	Adipose capsule of kidney
	Adjacent to adrenal gland
Residual lesion	Adjacent to external iliac artery
	Interstitial adipose tissue in tail of pancreas
	Pararectal adipose tissue
C. Intrathoracic adipose tissue	
Acute stage	Not observed
Intermediate stage	Adjacent to thoracic duct (upper part)
	Adjacent to thoracic duct (lower part)
Granuloma-like stage	Precordial mediastinal tissue
Residual lesion	Mediastinal and subepicardial adipose tissue
D. Adipose tissue examined ; no histological changes	
	Para-œsophageal adipose tissue near cardia
	Tissue in larynx (near vocal cord)
	Floor of mouth (between tongue and tonsil)

* Specimens which showed advanced lesions in some of the acini while others still presented the suppurative stage are included in the table as "acute stage" and marked "relapse".

single lobules entirely or in part replaced by granulomatous tissue, while other adjacent lobules had escaped completely. The following stages in the development of the granuloma-like lesions were found. (a) Polymorphonuclear leucocytes which had persisted were confined

Relapsing nodular panniculitis
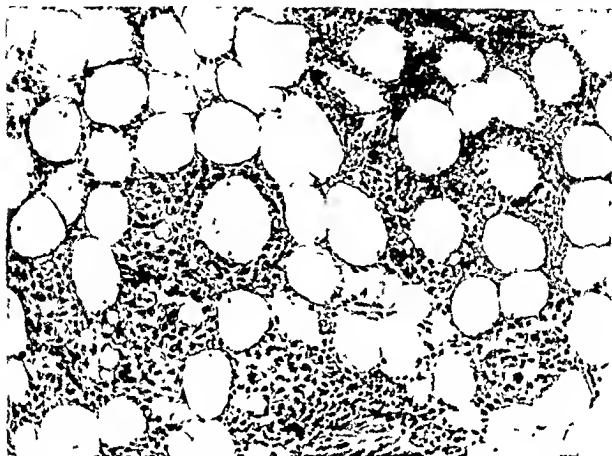
Fig 4 —Section of perirenal adipose tissue Upper right (near the centre of a lobule) a focus of subacute inflammation with necrosis still contains abundant polymorphonuclear leucocytes. The lower portion contains foamy cells progressing from a septum and from the adventitia of an artery towards the centro lobular region. ×125.
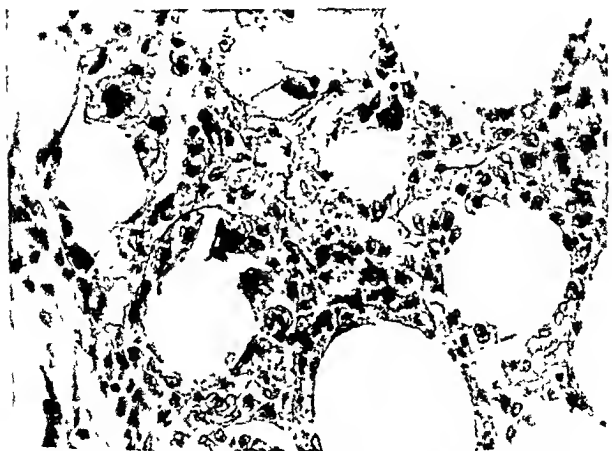
Fig. 5 —Section of adipose tissue from near the cisterna chyli So called "granuloma stage". ×325.

RELAPSING NODULAR PANNICULITIS
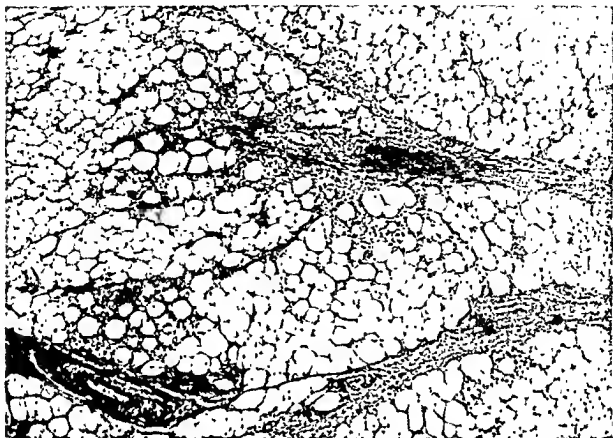
FIG. 6.—Section of the anterior mediastinal adipose tissue, showing the dependence of lesions on the distribution of septa. The upper half contains a fairly wide fibrous septum dividing into terminal septa. Limited by the latter, there are foci of inflammation in different stages of development. The "stem-septum" is free from infiltration in the extreme right of the picture; towards the terminal septa, increasing lymphocytic infiltration is seen. Even with low magnification it is possible to distinguish the close association of large foamy cells with the septa. ×55.
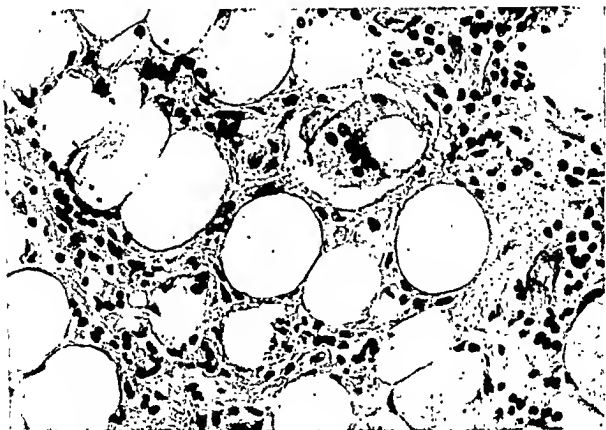
FIG. 7.—Residual lesion in adipose tissue adjacent to the lower portion of the thoracic duct. Fibrosis, scattered lymphocytes and isolated giant cells containing fat-droplets which stain with Ciaccio's method. ×330.

to the centre of the lobules (fig. 4). They appeared mixed with histiocytes and scattered giant cells but were never found to invade the interlobular fibrous septa. Giant cells of foreign-body type were seen also at the periphery of small cyst-like cavities, the presence of which within areas of acute inflammation has been shown above. Occasionally large vacuoles were seen in the cytoplasm of the giant cells.

The lesions described so far should be regarded as transitional between acute suppurative inflammation and the stage reported by many authers as " lipogranuloma ". In the fully developed granuloma-like stage the adipose tissue lobules were completely replaced by abundant foamy cells, in addition to other cells in varying numbers, including scattered lymphocytes (fig. 5), and there were hyperæmic capillaries which sometimes contained a few polymorphonuclears. This was the lesion which appeared when, clinically, the subcutaneous nodule reached the height of its development. It represents, therefore, the picture as seen in most biopsies and reported in many articles. It was seen in tho present case in a biopsy taken about 5 days after the first appearance of a nodule.

In sections where the process had not advanced far enough to obliterate the architecture of the adipose tissue, the granuloma-like lesions extended on the axis of the fibrous septa, as in the acute suppurative lesions. Then, however, tho changes commenced in the centres of lobules and advanced towards the septa, whereas the granulomatous change first became apparent close to the septa and gradually decreased towards tho lobular centres (fig. 6). Sometimes the granuloma-like changes involved the greater part of the lobules or even their entire area but they never extended beyond the limiting septa into adjacent lobules.

The extension of the lesions described was strikingly irregular. Next to lobules markedly involved were others which had remained intact. Still more striking was the presence of lobules in different stages of suppurative inflammation, separated by fibrous septa from lobules in the granulomatous stago of the process. The limitation of the lesions to individual lobules and tbe total absence of transgression from one lobule to the next strongly suggest tbat the morbid process did not spread continuously from one individual focus to another but was transmitted along the ramifications of the fibrous septa. The simultaneous appearance of lesions in different developmental stages within limited areas of adipose tissue allows one to assume that this transmission had occurred in successivo attacks. These have accordingly been called by us " relapsing inflammatory lesions ".

3. *Residual changes.* These were present in two different forms : (a) fibrous scars which contained only a few lymphocytes and rarely an isolated giant-cell (fig. 7) ; (b) foci of foamy-cells which did not include hyperæmic capillaries and were free from lymphocytic infiltration (fig. 8).

Specific stains for fatty substances. Benda's macroscopic reaction for fat-necrosis was negative in unfixed tissue in all specimens examined. With Ciaccio's method (after fixation in Orth's fluid) accumulations of disintegrating leucocytes were found to include extracellular granular sudanophil substance. In scattered foci of small histiocytes with eccentric dark nuclei, the cytoplasm was filled with sudanophil material, as were also the large vacuoles in the giant-cells. A distinct brownish-coloured network was present in the cytoplasm of the foamy cells. Fuchsinophil substance was nowhere in evidence.

Stains for bacteria. All blocks of adipose tissue from the abdominal cavity, femur and skin of the sacral region were stained for bacteria by the methods already mentioned, with negative results.

Lymph-nodes in retroperitoneal fat. These showed acute and chronic inflammatory changes. The fibrous capsule of nodes adjacent to acutely inflamed areas of adipose tissue contained numerous small round cells and fusiform cells with sudanophil particles in their cytoplasm. A considerable amount of fat was found in hypertrophied endothelial cells of the sinuses and within the medullary cells, especially those close to the sinuses, but also in the periphery of lymph follicles.

Blood vessels within the adipose tissue (other than the capillaries dealt with above). (a) In or near areas of inflammation the smaller arteries and veins showed hyperæmia and sparse adventitious foci of small round cells. Swelling and œdema of the vascular wall, reported by some authors in biopsies of panniculitis, were not seen by us in sections of Zenker-fixed material. (b) A single block of adipose tissue from the true pelvis contained two small arteries which were partially obstructed by organised thrombi. Inflammatory changes were not present in the vascular wall. (c) A section from a *cutaneous ulcer* in the sacral region contained a small artery showing leucocytic infiltration of the wall and recent thrombosis. The artery was situated within the adipose layer adjacent to the dermis and near an area of acute suppurative panniculitis. The arteritis was more marked towards the side of the panniculus adiposus, the inflammation extending continuously from the adipose tissue into the arterial wall and causing thrombosis (fig. 9). This was the only place where an extension of the suppurative lesion from the fat to any other tissue was found. (d) Blocks from the *iliac veins, superior hæmorrhoidal veins* and lower part of the *inferior vena cava* were cut, together with the surrounding adipose tissue. There were organised parietal as well as recent obstructive thrombi. No infiltration of the media was found, even where there was the most marked inflammation of the adventitial tissue.

RELAPSING NODULAR PANNICULITIS

FIG. 8.—Residual lesion from sub-epicardial adipose tissue. A small firm nodule is seen composed of foamy cells only. The free border (to left) is covered by intact epicardial mesothelium. × 60.

FIG. 9.—Acute panniculitis involving an arteriole near the dermis with resulting thrombosis. Ulceration was present in the overlying epidermis. × 160.

Discussion

Relation of clinical symptoms to anatomical lesions

The case here reported presented the known characteristics of the so-called Weber-Christian syndrome, namely, a nodular inflammatory lesion in the panniculus adiposus which regressed within a few days of its appearance, while similar lesions appeared in consecutive crops in all parts of the trunk and extremities. Simultaneously with the appearance of new nodules the temperature rose and there was general malaise.

Other findings, observed in some but not in all previously reported cases, were also present, namely, a chronic infective (streptococcal) focus (12 cases in the literature : Larkin *et al.*, 1944) ; acute swelling of the extremities (Bailey, 1937) ; rheumatoid symptoms in muscles and joints in the prodromal stage of the febrile attack (Bailey) ; positive serological reactions for syphilis, in spite of the absence of other evidence of the disease ; acute aggravation of symptoms following the use of iodine (or bromine) preparations (8 cases in the literature : Larkin).

Death was attributed to acute suppurative peritonitis due to haemolytic streptococci. After thorough examination of all structures covered by peritoneum, including the histological examination of the retroperitoneal adipose tissue, no local cause of the peritonitis could be found. We assume that the peritonitis was due to a haematogenous infection, most likely originating in the tonsils, in which streptococci were observed in sections.

Swelling of the lower extremities, observed during two relapses of the disease, was found to be due to recurrent thrombosis of the lower inferior vena cava and its large tributaries. Histological examination failed to reveal any anatomical cause of the thrombosis. There was no evidence of phlebitis despite the spread of inflammatory changes in the surrounding adipose tissue.

Post-mortem examination showed that inflammatory changes were not limited to the panniculus adiposus but were also present in varying intensity in the adipose tissues of the abdomen and thorax. Among the published cases of the Weber-Christian syndrome the involvement of adipose tissue outside the panniculus proper was mentioned only once—in the case recently reported by Spain and Foley. The presence of inflammatory foci in the corresponding areas may explain the repeated complaints by our patient of pain in the abdomen and behind the sternum, as well as the varying results of vaginal examination, where the pelvic wall was felt to be lined by nodular formations which repeatedly changed in size between the attacks.

Commentary on the lesions of the adipose tissue

Histological examination showed that the active morbid process presented itself, in different aspects, as a suppurative and as a

granulomatous inflammation. It ended either in fibrous scarring or as quiet focal foamy cell infiltration.

Shaffer (1938), who made a study of the histological changes in biopsy material from cases of the Weber-Christian syndrome, traced the first appearance of foamy cells and histiocytes near the fibrous septa of adipose tissue. Our own observations agreed with this finding but they did not confirm Shaffer's view, which was shared by Cummins and Lever (1938), and by Spain and Foley, that this represents the primary lesion.

As described above, the granulomatous lesions were preceded by stasis of polymorphonuclear leucocytes in the intralobular capillaries as well as by foci of suppurative inflammation in individual lobules. These changes were so constantly observed in our case that we have been inclined to view them not only as primary but also as the essential lesion of the disease.

The remarkable and unpredictable cropping-up of inflammatory foci in the adipose tissue in widely separated areas of the body suggests the effect of a blood-borne noxa. However, in spite of the presence of the tonsillar focus, a metastatic-infective process within the adipose tissue may with great probability be excluded. There were no bacteria in the adipose tissue lesions, and, while this fact may be explained by their rapid local destruction, it is difficult to understand why suppurative lesions due to streptococci should fail to extend beyond the confines of fibrous septa or adjacent parenchymatous organs. In fact these structures did not show even the least trace of leucocytic infiltration.

In our opinion, the non-suppurative changes have no specific significance in the pathological process of the Weber-Christian syndrome. They are more likely to be due to tissue injury by fatty substances liberated in the course of destruction of fat cells by suppurative inflammation (" coincidental panniculitis " ; Keil, 1935). In the present instance such substances could be demonstrated by staining with Ciaccio's method in histiocytes and giant cells or disseminated within necrotic areas. Their presence may also be inferred from the early appearance of foreign-body giant cells lining pseudocystic cavities formed by confluence of destroyed fat cells in areas of suppurative infiltration.

The histological findings in the fully developed subcutaneous nodule are those described in the literature under a variety of names, in accordance with the author's view concerning the origin of foamy cells—lipogranuloma, oleogranuloma, " Wucheratrophie " with or without accompanying chronic inflammation, lipophage granuloma. They are known to appear as a result of a great number of chemical, mechanical or thermal injuries or from vasomotor disturbances causing local ischemia. The non-specific character of the granulomatoid lesion is confirmed by investigations in the experimental animal (Franco, 1911) and in man (Goldzieher, 1931 ; Bogliolo, 1936).

Reports on multiple "lipogranulomas" by Russian pathologists (Abrikossoff, 1926, 1929) should be mentioned. The nodules were observed in obese individuals during the period of convalescence from infectious diseases, especially relapsing and typhus fevers. The lesions made their appearance predominantly in the panniculus adiposus, but also in the retroperitoneal adipose tissue, great omentum and mesentery. Although the original papers containing more extensive histological descriptions were not available, it appears from the summaries given by Abrikossoff that the Russian authors were referring to a pathological process in many respects similar to that seen in the Weber Christian syndrome.

SUMMARY

1 A fourth fatal case of the Weber Christian syndrome with post mortem findings is reported

2 Autopsy showed that the lesions were not limited to the panniculus adiposus but extended also to adipose tissue in the thorax and abdomen

3 Histologically the initial lesion was distinguished by leucocytic stasis in the blood capillaries and focal suppurative inflammation in the adipose tissue. It was followed by the development of granuloma-like lesions which arose on the axis of the fibrous septa. The process terminated in scar like formations or foci of foamy cell infiltration

4 The possible aetiology and the relationship between the different types of lesions are discussed

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A SUBSTANCE IN HUMAN SERUM INHIBITING STAPHYLOCOAGULASE

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The final identification of *Staphylococcus aureus* at present rests mainly on its ability to clot human plasma, originally observed by Loeb (1903-04) and Much (1908); the clotting agent (staphylocoagulase) may be obtained cell-free. The standard method of the coagulase test consists in adding some of a fluid culture of staphylococcus to plasma diluted ten times with saline. In the course of the routine examination of staphylococci an attempt was made to use more concentrated plasma; it was then found that some plasmas when undiluted failed to clot, even though they clotted well when diluted.

The possibility that there may be in the blood of certain human subjects, especially those suffering from staphylococcal infections, an antibody neutralising staphylocoagulase was first suggested by Kemkes (1928), who however failed to demonstrate it in the sera of five cases of chronic staphylococcal disease and several healthy individuals; live cultures of staphylococci were used for clotting. Gross (1931), incubating the sera first of all with cell-free coagulase, also failed to find anti-coagulase in the sera of rabbits immunised against staphylococci. Later, however (1933), he reported that certain human sera and anti-staphylococcal rabbit sera were capable of inhibiting staphylocoagulase up to a titre of 1 : 3. He also noticed that various samples of human plasma differed considerably in the time required for clotting by staphylocoagulase, some failing to clot. Sudhues and Schimrigk (1933), however, using live staphylococci, found no difference in clotting time between plasma derived from patients suffering from staphylococcal infections and that of healthy individuals. Walston (1935), despite negative results on attempting to immunise rabbits against coagulase, considered it to be antigenic and reported that certain anti-staphylococcal rabbit sera were capable of neutralising cell-free staphylocoagulase. On the other hand, Cruickshank (1937), using cultures of *Staphylococcus aureus* for clotting, failed to demonstrate anti-coagulase in commercial staphylococcal antibody or to find any difference in clotting time between the plasma of four patients suffering from staphylodermia and the plasma of healthy people. Finally, Smith and Hale (1944) reported that they were unable to immunise rabbits and guinea-pigs against cell-free staphylocoagulase, and they explained the occasional inability of human plasma to clot on the addition of coagulase as due to lack of a specific activating substance.

These contradictory results suggested the need of a more systematic investigation to ascertain whether (1) occasional samples of plasma or serum actually delayed or inhibited clotting by staphylocoagulase, and (2) the nature and mechanism of such a phenomenon.

The presence of an inhibitory substance in human plasma

Specimens of plasma (*ca.* 0·4 per cent. sodium citrate) from 104 persons, about equal numbers being healthy blood donors and patients suffering from various septic conditions, were examined in serial doubling dilutions with saline, ranging from 1 : 1 (undiluted) to 1 : 64. Clotting was induced by inoculating 0·5 c.c. with 2 drops of a 24 hours' broth culture of a known coagulase-positive *Staphylococcus aureus*. The tubes were then incubated for 24 hours at 37° C. Note was taken of the occurrence, rate of formation and extent of clot, grades of the latter being designated as follows : 0 = no clot, 1 = small floating ball, 2 = large sliding clot, 3 = almost solid, and 4 = completely solid clot. It was found that most of the plasmas clotted after 1½ hours at all dilutions ; certain plasmas, however, showed at this time no clotting in the strong concentrations (1:1 - 1:8) but clotted at higher dilutions. Clotting between 1 : 1 and 1 : 8 might take up to 24 hours and occasional plasmas failed to clot when undiluted. Assessment of clot at dilutions higher than 1 : 40 was difficult owing to the small amount of fibrinogen present. To obviate this and to avoid possible fallacies from progressively smaller amounts of fibrinogen receiving the same inoculum of staphylococci, the technique was altered as follows. The plasma of which the inhibitory power had to be tested was diluted with a 1 : 10 dilution of a readily coagulable plasma in saline instead of saline alone ; the fibrinogen content was thus almost constant in all tubes and the clot was easy to read. The results were identical with those previously obtained. It follows from these experiments that since certain plasmas which fail to clot at high concentrations do so when more dilute, and since the inhibitory property can be demonstrated in the presence of a well-clotting plasma, the phenomenon cannot be due to the absence of a factor such as the activating factor of Smith and Hale but is explicable only by the presence of an inhibitory substance. (The existence of the activating factor has incidentally been confirmed ; plasmas used in the present work, however, did not lack it.)

It was first necessary to make sure that the higher content of sodium citrate present in concentrated plasma as compared with dilute was not the inhibitory factor. This was tested by diluting the plasma with 0·4 per cent. sodium citrate solution in saline instead of saline alone. It was found that the concentrations of sodium citrate used were not responsible for the inhibition. Even 8 per cent. citrate did not interfere with clotting by formed coagulase, as present in a 24-hours' broth culture or in a cell-free preparation ; but 0·7-1 per cent. in the medium hindered its formation owing to inhibition of growth of staphylococci.

Next, by adding calcium chloride or thrombin to all plasmas which failed to coagulate under the influence of staphylocoagulase, it was

found that in general clotting occurred. Hence it was proved that the inhibitory substance acted specifically against staphylocoagulase clotting and did not affect the ordinary mechanism of blood clotting. The few plasmas which failed to clot on the addition of calcium or thrombin are not considered meanwhile.

So far, live cultures of *Staphylococcus aureus* were used to induce clot and it should be noted that differences observed between plasmas concerned chiefly the length of time required to effect clotting. It was thought that this procedure might not reveal fully the real differences between various plasmas, for if the inhibitory substance was capable of neutralising only a limited amount of coagulase, then the continuous production of the latter by living organisms would eventually overcome the inhibitory substance and cause clotting. This would account for the considerable delay, ending finally in clotting, observed with certain plasmas. Accordingly it was decided to use cell-free coagulase obtained by filtering highly active fluid cultures of *Staphylococcus aureus* (for details of preparation, etc., see below); the technique of the test was the same as before, a fixed amount of a well-clotting plasma being used along with serial dilutions of the inhibitory plasma. Fixed amounts of coagulase were added to the mixtures (from 20 to 80 times the minimal coagulating dose in different experiments). This method showed that very frequently plasmas which caused only delay in clotting by live cultures were capable of inhibiting entirely clot-formation by cell-free coagulase; many plasmas inhibited clot completely at dilutions between 1 : 2 and 1 : 6 and one at 1 : 40. Thus it became possible to express differences between plasmas in terms of their inhibitory titres instead of their clotting times. It follows from these experiments that a given amount of inhibitory substance is capable of neutralising only a fixed amount of coagulase; therefore the use of live cultures to induce clot tends to mask the true inhibitory titre of the plasma.

The presence of inhibitory substance in human serum

The hypothesis was then adopted that the inhibitory substance might be an antibody directed against staphylocoagulase, and an attempt was made to investigate its content in serum.

METHODS

Preparation of coagulase. A strain of *Staphylococcus aureus* producing highly active coagulase is grown for 24 hours at 37° C. in broth containing 10 per cent. plasma (0·4 per cent. sodium citrate) (Lominski, 1944). The culture is then shaken until the clot disintegrates; after preliminary filtration through paper the fluid is passed through a grade SB Ford's "Sterimat" and the sterility of the filtrate ascertained. (The filtrate keeps at −5° C. for several months without noticeable deterioration.) The minimal coagulating dose (M.C.D.) for a well clotting plasma is then determined, i.e. the smallest amount which in 24 hours at 37° C. causes a noticeable clot (grade 1) in 1 c.c. of 10 times diluted

plasma. This usually averages 0·001 c.c. (titre 1 : 1000). Coagulase obtained by filtration of cultures in ordinary broth without plasma has also been used; its titre was generally low (below 1 : 100); the results were identical with those obtained by the use of plasma-broth coagulase.

Plasma. Human plasma (0·4 to 0·7 per cent. citrate) was used throughout.

Sera were tested both fresh and after heating for 30 minutes at either 56° or 63° C. : they were derived from healthy individuals and patients suffering from various diseases.

The test. To tubes containing 0·5 c.c. of serial doubling dilutions of serum in saline (and to controls containing 0·5 c.c. saline or 0·5 c.c. of a known negative serum diluted four times) 0·2 c.c. of coagulase is added (diluted so as to represent 20-30 M.C.D.). After the mixtures have been incubated for 90 minutes at 37° C. each tube receives 0·3 c.c. of a 1 : 3 dilution of the same plasma as served for determining the M.C.D. Accordingly, the total volume in each tube is 1 c.c., the plasma being finally in a dilution of 1 : 10. The dilutions of serum shown in the tables are final dilutions.

Reading the test. Delay or absence of clotting in tubes containing mixtures of serum and plasma denotes the presence of the inhibitory substance. Controls usually begin to coagulate after 90 minutes and are solid after 2 hours. Readings may be made as soon as clotting has occurred in the controls or arbitrarily up to 24 hours, the highest dilution of serum which completely inhibits clot formation being noted.

RESULTS

The inhibitory substance was found to be relatively heat-stable, completely withstanding 56° or 63° C. for 30 minutes (heating at these temperatures does not render a negative serum inhibitory). However, fresh and heated sera differed in other respects as follows. Fresh sera in high concentrations (1 : 2 - 1 : 8) frequently clotted plasma *per se* even in presence of excess of sodium citrate, presumably owing to their content in thrombin, while certain other fresh sera in similar concentrations inhibited thrombin-clotting of plasma, presumably owing to antithrombin. The former property (thrombin) was removed by keeping for 3 or 4 days or heating for 30 minutes at 56° C., while the latter (antithrombin action) was removed by heating at 63° C. (compare Gasser, 1916-17, and Quick, 1938). Clotting of plasma, when caused by thrombin in fresh sera, interferes with the estimation of the inhibitory substance unless its titre is higher than the thrombin titre. The antithrombin property of sera was proved to depend on a factor different from the inhibitory substance (see below); it did not affect in any way the coagulase-neutralisation tests. Thus there was no need to heat sera to 63° C. ; and most of our tests were carried out with sera heated at 56° C.

In all, 348 sera have been examined, of which 212 contained inhibitory substance, to a titre of 1 : 1280 in 14, of 1 : 640 in 26, of 1 : 320 - 1 : 160 in 89, and of 1 : 80 - 1 : 8 in 83. The incidence of low-titre or non-inhibitory sera appeared higher among patients suffering from major staphylococcal infections than in those suffering from other diseases or among healthy people.

Relation of inhibitory substance to protein fractions of the serum

Twenty-five fresh sera showing a high content of inhibitory substance (titre over 1 : 160) were first fractionated with ammonium sulphate into albumin and globulin fractions and the latter subsequently into euglobulin and pseudoglobulin. Each fraction was made up with saline to the original volume of serum. Coagulase-neutralisation tests were then carried out with each fraction as described for whole serum. Table I shows that the globulin fraction

TABLE I

Showing the inhibitory substance content of various protein fractions of an inhibitory serum

Serum no. 27 and fractions	Serum dilutions								
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1.512
Whole serum . .	0	0	0	0	0	0	0	0	2
Globulin (50 per cent. sat. ammonium sulphate)	0	0	0	0	0	0	0	0	4
Euglobulin (28-33 per cent. sat. ammonium sulphate)	0	0	0	0	0	2	4	4	4
Pseudoglobulin (34-50 per cent. sat. ammonium sulphate)	0	0	0	0	0	0	0	1	4
Albumin (100 per cent. sat. ammonium sulphate)	0	4	4	4	4	4	4	4	4

In all tables the numerals designate the grades of clot (see p. 188) as read after 24 hours.

contained practically all the inhibitory substance. On further fractionation slightly more inhibitory substance came down with the pseudoglobulin than with the euglobulin. In the case of non-inhibitory sera none of the protein fractions showed the inhibitory property. Thus the inhibitory substance is either a serum globulin or very closely linked with it.

Specificity of action of the inhibitory substance on staphylocoagulase

It has already been mentioned that the inhibitory substance contained in plasma did not inhibit thrombin ; it has also been noted that in serum it was linked with the globulin fraction and was more heat-stable than antithrombin. Table II gives the results with a fresh serum showing both a high titre of inhibitory substance and strong thrombin inhibition. It shows that fractionation of serum proteins effects a complete separation of the two factors since, unlike the inhibitory substance, antithrombin is present in the albumin

fraction only (compare albumin X of Quick, 1938). Thus the inhibitory substance acts specifically on staphylocoagulase.

TABLE II

Relation of the inhibitory substance and antithrombin to serum protein fractions

Fresh serum no. 49 and fractions		Serum dilutions							
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
Whole serum	+coagulase	0	0	0	0	0	0	0	2
	+thrombin *	0	0	0	4	4	4	4	4
Albumin fraction	+coagulase	2	4	4	4	4	4	4	4
	+thrombin *	0	0	4	4	4	4	4	4
Globulin fraction	+coagulase	0	0	0	0	0	0	2	4
	+thrombin *	4	4	4	4	4	4	4	4

* 0·5 c.c. full strength thrombin (prepared according to Quick's (1942) method) was added to 0·5 c.c. of the various dilutions of serum and the mixtures incubated at 37° C. for 90 minutes; 0·1 c.c. of undiluted plasma was then added.

Mechanism of action of the inhibitory substance

It is striking that in contrast with the frequent presence of the inhibitory substance in serum, only a few of the 104 plasmas examined inhibited clotting by coagulase, and this only when undiluted. Clearly, if plasma were as frequently and as strongly inhibitory as serum the coagulase reaction could not be used as a routine test. It seemed unlikely that this contrast was due to the sera representing a selected population differing from that yielding the samples of plasma, but to exclude this possibility the inhibitory titres of plasma and serum from the same individuals were determined. To this end five plasmas were selected which did not inhibit clotting at any concentration but which delayed clotting in higher as compared with lower concentrations. Part of each sample was re-calcified, the serum collected and its inhibitory substance content estimated; the inhibitory titre was 1:80 with 1, 1:40 with 3, and 1:20 with 1. Thus a significant difference was established between the inhibitory power of plasma and serum of the same individual. Hence either the inhibitory substance must be formed during conversion of fibrinogen into fibrin or else its activity in plasma is masked. The second possibility appeared the more likely. It was thought that the inhibitory substance existed both in plasma and in serum, but that its inactivity in the former might be due to coagulase having a greater affinity for fibrinogen than for inhibitory substance (compare Quick's view as to the greater affinity of thrombin for fibrinogen than for antithrombin). Thus if staphylocoagulase be presented with fibrinogen and inhibitory substance simultaneously, it will react with the former and clot it. If, on the other hand, fibrinogen is absent,

as in serum, coagulase incubated with inhibitory substance becomes neutralised, and fibrinogen added subsequently can no longer be clotted.   To test this the following experiments were carried out. Twelve sera were examined, three sets of serial doubling dilutions being set up for each: (*a*) serum was incubated for 90 minutes with coagulase (50 M.C.D.) and then plasma added ; (*b*) serum, coagulase and plasma were mixed simultaneously, and (*c*) serum was first incubated for 90 minutes with plasma and then coagulase added. Table III gives an example of the results obtained.

<center>TABLE III</center>

Showing the relative affinity of staphylocoagulase for fibrinogen and for the inhibitory substance

Serum no 32	Serum dilutions							
	1 2	1 4	1 8	1 16	1 32	1 64	1 128	1 256
Serum + coagulase incubated, then plasma added	0	0	0	0	0	0	0	1
Serum, coagulase and plasma mixed simultaneously	1	4	4	4	4	4	4	4
Serum + plasma incubated, then coagulase added	1	4	4	4	4	4	4	4

The results show that if fibrinogen is present when coagulase and inhibitory substance are mixed the effect of the latter is almost nil. Clearly, therefore, coagulase has a greater affinity for fibrinogen than for the inhibitory substance and for this reason the investigation of plasma gives practically no evidence of the latter's activity. It also follows from these experiments that the inhibitory substance acts directly on staphylocoagulase and not by rendering plasma non-coagulable.

Quantitative relations between the inhibitory substance and coagulase

This was investigated by testing the neutralising power of serum dilutions for increasing amounts of coagulase, while the fixation time (incubation of coagulase-serum mixture) remained the usual 90 minutes. The results (table IV) indicate that under the conditions of the experiment, time being constant, a fixed amount of the inhibitory substance is capable of neutralising only a fixed amount of coagulase (see also p. 189). The rule of multiple proportions holds fairly well.

Influence of fixation-time on the inhibitory titre of sera

In these experiments the amount of coagulase was kept constant (25 M.C.D.) but the time allowed for neutralisation of coagulase by

the inhibitory substance ranged from 0 to 360 minutes. From the results (table V) it is seen that the neutralisation of coagulase by the inhibitory substance does not occur instantaneously and does not progress indefinitely with increased fixation-time.

TABLE IV

Quantitative relations between the inhibitory substance and coagulase

Coagulase	Serum N : dilutions								
	1 : 10	1 : 20	1 : 40	1 : 80	1 : 160	1 : 320	1 : 640	1 : 1280	1 : 2560
1 M.C.D. . .	0	0	0	0	0	0	0	0	3
5 ,, . .	0	0	0	0	0	0	4	4	4
10 ,, . .	0	0	0	0	0	1	4	4	4
20 ,, . .	0	0	0	0	0	4	4	4	4
50 ,, . .	0	0	0	1	3	4	4	4	4
100 ,, . .	0	0	3	4	4	4	4	4	4

TABLE V

Influence of fixation-time on the inhibitory titre of sera

Fixation-time in minutes	Serum no. 73 : dilutions								
	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128	1 : 256	1 : 512
0	1	3	4	4	4	4	4	4	4
30	0	1	4	4	4	4	4	4	4
60	0	0	0	3	4	4	4	4	4
90	0	0	0	0	0	0	1	3	4
120	0	0	0	0	0	0	0	1	4
240	0	0	0	0	0	0	0	1	4
360	0	0	0	0	0	0	0	1	4

Reversibility of the coagulase-inhibitory substance

Serial doubling dilutions of an inhibitory serum were set up in the usual way. After 24 hours at 37° C. the tube was selected which contained the highest serum dilution showing no clot, and its contents divided in two. Half was kept for a further 24 hours at 37° C., while to the other half 9 volumes of plasma diluted 1 : 10 were added and the tube replaced in the incubator. The undiluted tube, serving as a control, remained unclotted, whereas clot formed in the tube to which further plasma—*i.e.* fibrinogen—had been added. Thus the inhibitory substance is capable of neutralising coagulase without bringing about a lasting loss of all its clotting power.

DISCUSSION

There are marked discrepancies between the findings of previous workers, even when positive, and the present results. Several factors

may account for these differences.' Thus Kemkes (1928), Sudhues and Schimrigk (1933) and Cruickshank (1937), who all used live cultures of staphylococci to induce clotting, failed to observe inhibition of coagulase. This is easily explicable if one considers that a fixed amount of inhibitory substance is capable of neutralising only a limited amount of coagulase; with live cultures producing ever increasing amounts of coagulase, results were bound to be nil or nearly so. Gross (1933) and Walston (1935), who detected coagulase-neutralising properties in both human serum and anti-staphylococcal rabbit serum, found extremely low titres as compared with our results. It must be noted, however, that in their experiments the time of contact was shorter (60 minutes); now, as has been seen, the time allowed for fixation between the inhibitory substance and coagulase is a decisive factor. Also data are lacking as to the amounts of coagulase used by them; here again, our experiments show that if there is an excess of coagulase relative to the inhibitory substance no information about the latter may be obtained.

Another problem is the nature of the inhibitory substance; many of the findings are in favour of its being an antibody. The following properties, generally accepted as characteristic of antibodies, are shown by it :—linkage with the globulin fraction of serum proteins, relative heat-resistance, specificity for the corresponding antigen, reaction with the latter following the rule of multiple proportions, and time-relations according to which antibody combines gradually with the antigen but cannot neutralise more than a fixed amount of antigen no matter how long the time of contact. By definition, however, an antibody is an immunity response to an antigen; there is actually little evidence, and that conflicting, as to whether coagulase is antigenic. Thus Gross (1933) was able to produce in the rabbit an immune anti-coagulase, but both Walston (1935) and Smith and Hale (1944) failed. Walston attempted to immunise two rabbits by intraperitoneal and intravenous injections and despite negative results considers coagulase as antigenic; Smith and Hale give no details of their experiments. The failures may be due to such factors as mode of immunisation and insufficient length of treatment, or rabbits and guinea-pigs may be less responsive than man. Although the possibility cannot be entirely excluded that the inhibitory substance is not an immunity response to staphylococcus and only by chance exhibits so many properties of an antibody, nevertheless the positive results of immunisation obtained by Gross (1933) and our in-vitro observations appear more significant than the failures. Accordingly, the antibody hypothesis, since it fits most of the observed facts, may justifiably be maintained until experimental proof to the contrary is brought. The scantiness of the inhibitory substance in commercial antitoxic rabbit sera may then be explained on the following lines. Gross (1931-32) found coagulase only in filtrates of young cultures (we can amply confirm this) and when hæmolysin or toxin was present coagulase

could no longer be found. Since highly toxic preparations of old cultures are generally used for the purpose of obtaining antitoxic sera, absence or low titre of a coagulase-neutralising antibody in antitoxic rabbit sera is to be expected (see also Walston).

In the present study no serious attempt has been made to establish the antigenicity of coagulase by correlating the serological findings with the clinical condition of the individuals yielding the sera. It can be said, however, that the incidence of high titres of inhibitory substance in the sera of clinically healthy people or patients with non-staphylococcal diseases is considerably greater than among patients suffering from severe staphylococcal infections. On the view that the inhibitory substance is an antibody, its absence or presence only in low titre in a serum would appear to predispose to major staphylococcal infections. Its presence in the blood of clinically healthy people (considering the extensive natural distribution of *Staphylococcus aureus*) could be accounted for by previous staphylococcal infections or silent immunisation, or by its being a so-called natural antibody. Successful results of experimental immunisation with coagulase in man or animals should definitely determine the nature of the inhibitory substance.

SUMMARY

1. A substance specifically inhibiting the clotting of plasma by staphylocoagulase was found in human sera; it acts by neutralising coagulase and not by rendering plasma non-coagulable. A technique for its quantitative estimation is described.

2. The inhibitory substance was found in 212 out of 348 sera; its incidence in healthy people appears to be higher than among patients suffering from severe staphylococcal infections. No attempt was made, however, to correlate closely clinical with serological findings.

3. The inhibitory substance is precipitated by ammonium sulphate with the globulin fraction of serum proteins and is relatively heat-stable, resisting 63° C. for 30 minutes.

4. It is different from Quick's albumin X, which inhibits thrombin clotting of plasma.

5. Neutralisation of coagulase by the inhibitory substance requires time, but neutralisation does not progress indefinitely with increase of time. The coagulase-inhibitory substance relation conforms to the rule of multiple proportions. The reaction is, in part at least, reversible.

6. The inhibitory substance has antibody characteristics but cannot yet be definitely accepted as such.

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THE LESIONS OF SHEEP DERMATITIS VIRUS INFECTION IN THE RABBIT AND GUINEA-PIG WITH PARTICULAR REFERENCE TO THE TUMOUR VIRUSES *

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(PLATES XXII-XXVI)

THE transmission to rabbits and guinea-pigs of a virus derived from an outbreak of sheep dermatitis has been described in two previous communications (Selbie, 1944, 1945). Inoculation of the virus on the scarification lines produces in the rabbit a pseudopapillomatous lesion, whereas in the guinea-pig the lesion is of a more exudative type. The infection in the guinea-pig is also of shorter duration and is followed by less resistance to reinfection than in the rabbit. In this communication the histology of the rabbit and guinea-pig lesions are described and the significance of the difference in the response of these animals to the virus is discussed.

The rabbit lesion

First week. In rabbits that have been inoculated with fully active virus suspensions the only visible reaction during the first 6-9 days is the healing of the scarification lines, which is usually completed by the 3rd or 4th day.

Histology. On microscopical examination changes are already apparent between the 4th and 7th days, or 2 days before there is any naked eye evidence of infection. At this early stage there is some thickening of the epidermis, especially the malpighian layer, where the cells are slightly enlarged and increased in number. That this change is hyperplastic in nature is evident from the increased number of mitoses in the basal layer. There is also a moderate infiltration of the most superficial part of the dermis by mononuclear inflammatory cells.

Second week. The first visible evidence of infection, which usually appears at the beginning of the second week, is the presence of slightly scaly erythematous patches which become confluent and more deeply erythematous within the next 2 days. Meanwhile scaliness increases and some thickening of the skin can be detected. By the end of the second week the skin is considerably thickened and is covered with an opaque layer of dry scales.

Histology. A section of a lesion 9 days after inoculation and 3 days after erythema and scaliness first appeared shows that epithelial hyperplasia has progressed considerably and that the scaly appearance of the lesion is due to parakeratosis (fig. 1). Epithelial hyperplasia is no longer confined to the epidermis but has now extended to the upper parts of the hair sheaths. Inflammatory infiltration by mononuclear cells is still moderate and largely confined to the superficial part of the dermis, while congestion and œdema are now apparent, particularly in the dermal papillæ.

By the end of the second week hyperplasia is much more evident in the upper parts of the hair sheaths than in the epidermis (fig. 2). There is now considerable congestion and œdema of the dermis, while the inflammatory cellular infiltration is more intense and extends to a greater depth, although it is still confined to the immediate neighbourhood of the affected hair follicles. Under higher magnification it can be seen that, whereas in the earlier stages the inflammatory cells are mainly large mononuclear cells with pale nuclei, there is now, in addition, an infiltration by an almost equal number of small lymphocytes and a few polymorphonuclear leucocytes.

Third week. During the third week after inoculation the thickening and scaliness of the skin increases progressively, so that the lesions become nodular, with a rough, scaly, almost warty surface. Towards the end of the week it becomes apparent that the increased nodularity is in part due to small abscesses from which pus can be readily expressed. This pyogenic infection combined with the virus lesion produces characteristic scurfy nodules which are covered with a mass of matted hair, keratin scales and dried exudate.

Histology. Microscopical examination of a lesion 19 days after inoculation shows great hyperplasia of the hair sheaths, which have extended to the surface to form a series of papilliform outgrowths covered with irregular layers of keratin. Here again hyperplasia is confined to the upper parts of the hair follicles and the inflammatory reaction is mainly associated with the hyperplastic epithelium.

Fourth week. The course of the experimental disease during the fourth week depends largely on the severity of the pyogenic infection and apparently to some extent on the sensitivity of the rabbits. In most rabbits the nodular growths continue to increase in size so that the lesions look like scurfy papillomatous growths with irregular tufts of hair.

Histology. The effects of pyogenic infection have now become prominent, especially in the hyperplastic hair follicles, which are readily infected by bacteria and trichophyton. In fig. 3, from a lesion 24 days after inoculation, it can be seen that a hyperplastic hair follicle is distended with pus which has broken through the lining epithelium to form an abscess in the neighbouring tissue. However, in spite of the superimposed pyogenic infection, the hyperplastic character of the virus lesion is well maintained and at a higher magnification (fig. 4) numerous mitoses can be seen in the proliferating epithelium. The supporting connective tissue is highly congested and œdematous and is heavily infiltrated, mainly by mononuclear cells and lymphocytes. Even at this stage polymorphonuclear leucocytes are not found in any number except in association with the follicular abscesses.

Subsequent course. The lesions are usually fully developed by the end of the fourth week and in most rabbits remain substantially unchanged for a further 2-4 weeks. During regression, which may commence at any time from the 4th to the 8th week, the superficial nodules drop off, leaving a thickened and slightly scaly epithelium with small ulcers which heal rapidly.

Histology. A section of a lesion 32 days after inoculation shows that the massive hyperplasia of the hair follicles has formed a papillomatous type of growth. This is heavily infected, but the collections of pus are mostly contained within enlarged hair follicles. The follicular outgrowths are supported by a

PLATE XXII

SHEEP DERMATITIS

FIG 1 —Rabbit 119, 23rd rabbit passage, 9 days after inoculation with an extract of fresh rabbit lesions and 3 days after first naked eye evidence of infection Hyper plasia of epidermis and upper parts of hair sheaths, moderate mononuclear infiltration of dermis   H and E   ×95
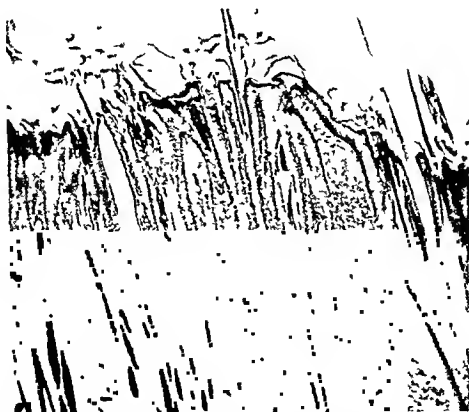
FIG 2 —Rabbit 51, 8th rabbit passage, 14 days after inoculation with an extract of fresh rabbit lesions and 7 days after first naked eye evidence of infection Hyper plasia now more advanced in hair sheaths than in epidermis, congestion, œdema and inflammatory infiltration of dermis   H and E   ×28

highly vascular connective tissue which is heavily infiltrated, mainly by small lymphocytes. Large masses of lymphocytes are also found in what appear to be dilated lymphatic channels, especially near the tips of the papilliform outgrowths. Large mononuclear cells with pale nuclei are also present in large numbers and form the majority of the infiltrating cells towards the base of the lesion, while plasma cells are found in small numbers, especially in association with degenerating epithelium. It is again noteworthy that only small numbers of polymorphonuclear leucocytes are found in the connective tissue, except where the epithelial lining of infected hair follicles has been destroyed.

The papillomatous appearance of the lesion persists for a variable period, during which the follicular abscesses increase in size so that the outgrowths finally consist mainly of multiple abscesses lined by hyperplastic epithelium which is in turn supported by narrow tongues of connective tissue. Even when the lesions are beginning to separate a moderate number of mitoses are found in the hyperplastic epithelium, while the inflammatory cells infiltrating the connective tissue are still mainly large mononuclear cells, lymphocytes and plasma cells, with a relatively small number of polymorphonuclear leucocytes.

The areas of ulceration left by the separation of the superficial infected mass are rapidly covered by epithelium. Deep extensions of the follicular abscesses into the dermis are also rapidly resolved, so that within 10 days all that remain are foci of degenerating epithelium, macrophages, lymphocytes, plasma cells and a few polymorphonuclear leucocytes. The skin may take as long as 3 weeks to regain its normal histological appearance; even then there is still some irregularity in the arrangement of the regenerated hair follicles and some infiltration by mononuclear inflammatory cells immediately under the epidermis.

The guinea-pig lesion

First week. In guinea-pigs that have been inoculated with active virus material specific changes in the skin do not become visible until at least the 6th day after inoculation, when a few minute scattered reddish brown papules appear.

Histology. The first histological evidence of infection can be seen 3 days after inoculation, when the epidermis shows some enlargement of the malpighian cells and increased mitotic activity in the basal layer. At a few scattered points of the epidermis there is also a widening of the spaces between the malpighian cells characteristic of intercellular oedema (fig. 5). These epithelial changes are accompanied by a moderate infiltration by mononuclear cells immediately under the epidermis.

Within the next two days there appear small foci in the epidermis where oedema has progressed to form a number of locules between the epithelial cells, which are themselves distorted by the pressure of the intercellular fluid (fig. 6). These locules contain mononuclear inflammatory cells which have apparently invaded the intercellular spaces by way of breaches in the basal layer as shown towards the right of fig. 6.

Second week. On the 7th or 8th day the inoculated area of skin begins to get scaly in patches and papules can no longer be detected. Within two days the whole area is deeply erythematous, thickened and covered with a layer of fine scales. This is followed by an exudative phase, so that by the end of the week the affected skin is covered with a flat brittle scab consisting of scales and dried exudate.

Histology. Microscopical examination at the beginning of the second week shows that the epidermis is considerably thickened, there being numerous mitoses in the basal layer and hypertrophy of the malpighian cells. Intercellular oedema is present in the basal layer and has progressed to the formation of a few scattered intra-epidermal vesicles in the malpighian layer. There is

also commencing hyperplasia and intercellular œdema in the hair sheaths. The dermis is congested but shows only a moderate infiltration by mononuclear inflammatory cells.

An advanced vesicular lesion is shown in figs. 7 and 8, from a section of skin removed 11 days after inoculation. This lesion consists of a pustule superimposed on a multilocular vesicle from which it is divided by an irregular layer of keratinised cells (fig. 7). The vesicle is traversed by a hair follicle to the right of which the basal layer of the epidermis has disappeared. This part of the vesicle contains a network of partially detached epithelial cells, inflammatory cells and a few strands of keratin. The part of the vesicle to the left of the hair follicle is shown at a higher magnification in fig. 8. The vesicle here consists of a number of locules of various sizes, the walls of which are epithelial cells which have been stretched and flattened out. Comparison with fig. 6 will show that this has been brought about by the increasing intercellular œdema which, at first producing locules between the cells, goes on to form larger locules by stretching and disrupting the epithelial cells. The contents are degenerating epithelial cells, mononuclear inflammatory cells and a few polymorphonuclear leucocytes. Other changes seen at this stage are intercellular œdema and invasion by mononuclear cells of the hyperplastic upper parts of the hair sheaths and moderate infiltration of the dermis by mononuclear cells and a few lymphocytes and polymorphonuclear leucocytes.

This process of vesiculation continues until the upper part of the epidermis, including a large part of the malpighian layer, sloughs off, as shown in fig. 9 of a section of skin 12 days after inoculation. The slough consists of keratin, keratinised and degenerating epithelial cells, large numbers of polymorphonuclear leucocytes and exudate. The remaining epithelium, including that of the superficial parts of the hair sheaths, is hyperplastic and œdematous, and the intercellular spaces are invaded by numerous polymorphonuclear leucocytes and a few mononuclear inflammatory cells. The dermis immediately below the epithelium is congested, œdematous and moderately infiltrated by mononuclear cells and to a much less extent by polymorphonuclear leucocytes.

Subsequent course. During the greater part of the 3rd week there is little change in the general appearance of the lesion except that the scab gets thicker and shows numerous fissures which have a tendency to bleed. Towards the end of the 3rd week the scab begins to flake off, leaving an entire, somewhat thickened and hairless skin by the end of the 4th week.

Histology. The degenerative changes in the epithelium are of short duration and are followed by a phase of continuing hyperplasia of the upper parts of the hair sheaths. The affected epithelium is still œdematous and infiltrated by numerous polymorphonuclear leucocytes, while the dermis is congested and infiltrated by a moderate number of mononuclear cells and polymorphonuclear leucocytes.

Resolution of the lesion is now rapid, so that by the end of the 3rd week the skin has in most cases almost regained its normal appearance, except that the malpighian layer is considerably wider than normal and numerous mitoses are present in the basal layer (fig. 10). In some cases hyperplasia of the hair sheaths continues for a somewhat longer period but is never as prolonged or as extensive as that found in the rabbit. Congestion of the dermis, with a moderate inflammatory cell infiltration, mostly by mononuclear cells, is still present at the end of the 4th week and the skin may not entirely regain its normal histological appearance until about the 6th week after inoculation.

Discussion

It has already been shown (Selbie, 1944) that the virus agent of this experimental disease was derived from an infectious outbreak in

lambs, which, in its epidemiological, clinical and histological features, was characteristic of contagious pustular dermatitis of the sheep as described by Glover (1928). Neutralisation tests on rabbits with the sera of lambs recovered from infection with known strains of the natural sheep virus also indicate that the experimental virus is closely related to the sheep virus, but in view of a recent failure to transmit the experimental virus to lambs there is still some doubt of its identity (Selbie, 1945). The histological features described here, however, give further evidence of the identity of the experimental virus with known strains of the sheep virus in that the guinea-pig and rabbit lesions closely resemble the two forms of the sheep virus infection described by Glover. The guinea-pig infection is similar to the lamb infection following inoculation on the inner aspect of the thigh, which commences as papules on the fifth day and, after passing through the stages of vesicle, pustule and scab, terminates with the scab falling off about the 24th day. Microscopically the guinea-pig and lamb lesions show the same stages of vesicle, pustule and slough, although the lamb lesion is perhaps somewhat more degenerative, with a greater preponderance of polymorphonuclear leucocytes in the inflammatory infiltration. The rabbit infection, on the other hand, is similar to that following inoculation on the lip of the lamb, in which an exudative stage lasting up to the 15th day is followed by a phase of epithelial proliferation leading to the formation of dense papillomatous growths. This phase persists for several weeks. Another respect in which these conditions resemble each other is that virus inclusion bodies have not been observed in the infection described here nor in contagious pustular dermatitis (Glover). The histological findings are thus consistent with the view that the experimental virus strain is closely related to known strains of the sheep virus and differs only in the range of its pathogenicity to animals.

The diversity of the lesions produced by this virus are of considerable interest in relation to the pathology of virus infections generally. Borrel (1903), in his account of the epithelioses and epitheliomata, held that the primary response of epithelial cells to the action of a virus is proliferative and that the epithelial virus diseases can be roughly classified into groups depending on whether they proceed to the formation of pustules or epithelial overgrowth. However, the experimental infection here described shows that a single virus strain can produce infections belonging to both Borrel's main classes. In the guinea-pig the infection is of a degenerative type, with formation of vesicles and pustules and destruction of most of the epidermis, whereas the rabbit lesion is of a proliferative type, with hyperplasia of the hair follicles leading to papillomatous outgrowths which may persist for as long as 6-8 weeks after inoculation. At the same time the lesions show a certain affinity in that there are proliferative changes in the guinea-pig, particularly in the hair sheaths, but to a much less extent than in the rabbit.

It has already been reported that the rabbit is completely refractory to reinfection with this virus for at least 100 days after successful primary inoculation, whereas the reinfected guinea-pig responds with a slightly modified infection (Selbie, 1945). The diversity of the lesions in these animals may therefore be due to the development of resistance being more complete in the rabbit than in the guinea-pig, thus determining a less virulent infection and so a more proliferative type of response. In support of this view is the production of degenerative lesions in young animals with the viruses of fowl lymphomatosis, fowl tumours and rabbit fibroma. Blakemore (1939) has inoculated young chicks with a virulent strain of fowl lymphomatosis virus and has obtained necrotising inflammatory lesions in the heart and liver followed by neoplasia in some of the animals that survive. When Rous or Fujinami fowl sarcoma virus is injected into newly hatched chicks (Duran-Reynals and Thomas, 1940-41) or chick embryos (Milford and Duran-Reynals, 1943) it causes, instead of tumour growth, a destructive hæmorrhagic disease, a response which has been ascribed by Duran-Reynals and Estrada (1940) to a lack of resistance to the virus. Similarly the rabbit fibroma virus induces in adult rabbits a neoplastic type of lesion of considerable duration, but in new-born rabbits an acute, predominantly inflammatory and destructive disease, which again can be correlated with low resistance to the virus (Duran-Reynals, 1940-41, 1945).

Factors other than general resistance, however, may also play a part in determining the response to a virus infection. This can be seen in the diverse lesions caused by a single virus in different situations in the same host. One example, that of the degenerative and proliferative lesions of contagious pustular dermatitis on the thigh and lip of the lamb, has already been mentioned. Another example is in sheep-pox, where the visceral lesions are highly hyperplastic in contrast to the exudative lesions of the skin. In these infections it would appear that the response to the virus, or perhaps to immune bodies, is determined by some intrinsic cellular factor. Such a factor would also appear to play a part in determining the response of the guinea-pig and rabbit to sheep dermatitis virus, because the distinctive degenerative character of the guinea-pig infection is maintained even in the presence of partial immunity, which would be expected to alter the nature of the lesion if immunity was the only determining factor.

The findings here described and those quoted from other workers have an immediate interest in relation to the tumour viruses because they also are highly dependent on the susceptibility of the host cells. Thus the papilloma virus of Shope and Hurst (1933) in its natural host, the cotton tail, causes a benign papillomatosis which is usually self-limited and rarely becomes malignant, but when this virus is transferred to the domestic rabbit it causes a much more progressive infection which almost invariably terminates in carcinoma. Such an outcome can be

SHEEP DERMATITIS
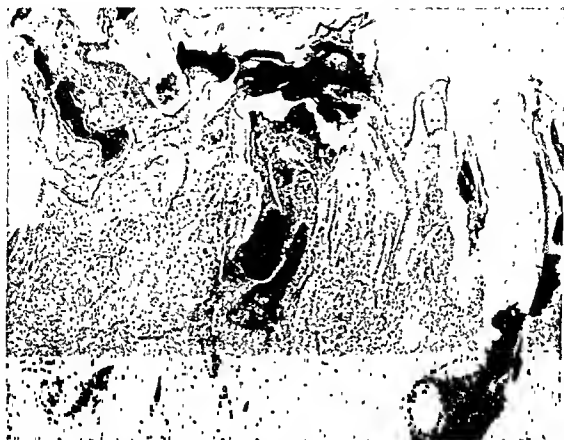
FIG. 3.—Rabbit 36, 7th rabbit passage, 24 days after inoculation with an extract of fresh rabbit lesions and 15 days after first naked eye evidence of infection. Outgrowth of hyperplastic hair follicles with pyogenic abscess formation. H. and E. ×21.
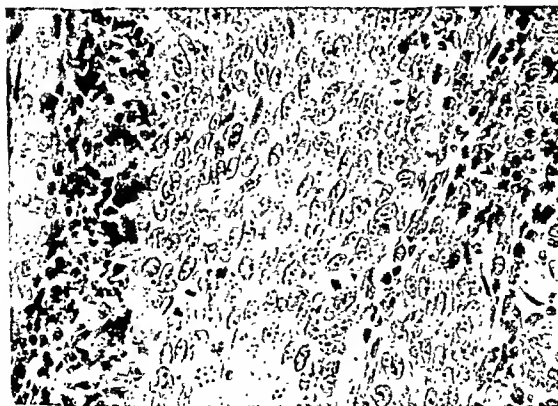
FIG. 4.—Higher magnification of epithelium towards the right of fig. 3 to show mitotic figures. ×385.

SHEEP DERMATITIS

Fig 5 —Guinea pig 11, 3 days after inoculation with an extract of dried lesions from rabbits 89 and 90 that had been stored for 100 days Intercellular œdema of epidermis, moderate mononuclear infiltration of dermis H and E ×280

Fig 6 —Guinea pig 11, 2 days later than in fig 5 and 1 day before first naked eye evidence of infection Intra epidermal locules containing mononuclear inflammatory cells H and E ×390

SHEEP DERMATITIS

Fig 7—Guinea pig 13, 2nd guinea pig passage, 11 days after inoculation with an extract of fresh lesions from guinea pigs 11 and 12 and 4 days after first naked eye evidence of infection   Pustule superimposed on multilocular intra epidermal vesicle. H. and E.   ×85
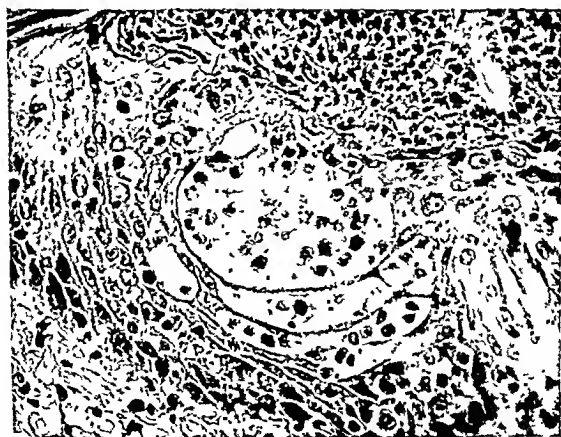
Fig 8—Higher magnification of part of vesicle in fig 7, showing locules separated by distorted epithelial cells   ×390

SHEEP DERMATITIS
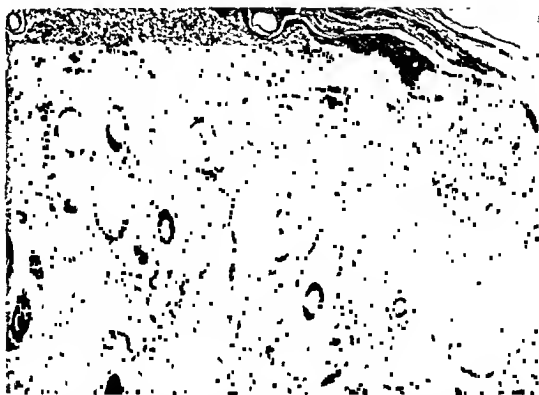
FIG. 9.—Guinea pig 9, 12 days after inoculation with an extract of dried lesions from rabbits 83 and 84 that had been stored for 184 days and 5 days after first naked eye evidence of infection. Necrosis and separation of upper layer of epidermis H. and E. ×85.

FIG. 10.—Guinea-pig 9, 7 days later than in fig. 9. Resolution of the lesion. H. and E. ×85.

greatly accelerated by preliminary painting of the skin with a carcinogenic agent (Rous and Kidd, 1938 ; Kidd and Rous, 1938 ; McIntosh and Selbie, 1940). In like manner the activity of the milk factor, which has been shown by Bittner (1937) to be the essential agent in the genesis of mammary cancer in certain strains of mice, is profoundly modified by differences in the hereditary constitution or hormonal stimulation of the mammary tissue of the host (Bittner, 1941 ; Dmochowski and Gye, 1944 ; Dmochowski, 1944). The same is true of the fowl sarcoma viruses, which can be greatly favoured in their activity by the provision of a suitable tissue reaction as in the Rous sarcoma virus (McIntosh and Selbie, 1943) or by the choice of susceptible strains of fowls for the chemical induction of tumours and their propagation by cell-free filtrates (McIntosh, 1933 ; McIntosh and Selbie, 1939). In this connection the observations of Duran-Reynals and Thomas (1940-41) on the purely degenerative lesions caused by Rous and Fujinami sarcoma viruses in young chicks are of particular interest in that they show that tumour viruses are capable of producing lesions other than neoplastic.

The activity of tumour viruses is thus so highly conditioned by environmental influences that it would almost appear that these agents play only a lesser role in tumour production. However, it has been shown here that a high degree of dependence on the nature and environment of the infected cells is not a peculiarity of the tumour viruses and cannot therefore be adduced as evidence against the essential role of these agents in the genesis of virus tumours.

Summary

The histology of rabbit and guinea-pig lesions produced by a virus derived from an outbreak of sheep dermatitis is described.

The characteristic feature of the lesion in the rabbit is an excessive hyperplasia of the hair sheaths, which form large papillomatous outgrowths, whereas in the guinea-pig the lesion is of a degenerative type similar to vaccinia.

The significance of the difference in the response of these animals to the virus is discussed with particular reference to the tumour viruses.

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THROMBOSIS AS A FACTOR IN THE PATHO-GENESIS OF CORONARY ATHEROSCLEROSIS

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(PLATES XXVII-XXX)

WE have been taught to regard atherosclerosis as an overgrowth of and degenerative change in the intima of arteries. This view is so firmly established that we have come to regard it as a ruling principle, yet evidence may be found in the coronary arteries that the lesions we classify as atherosclerosis can arise by quite a different process, namely the organisation of thrombus. For a number of years sections of a thrombosed coronary artery were used in our pathology classes to illustrate the process of canalisation. The artery, which had an organised thrombus with many channels running through it, provided a large number of sections, until a point was reached where the formation changed. Instead of many channels there was now a single lumen surrounded by a greatly thickened wall in which were foci of fatty degeneration. The picture had in fact changed from thrombosis to atherosclerosis. In order to see how this had come about, the sections were examined in serial order, whereupon it was found that the thrombosis was not limited to the region in which there was canalisation, but extended further along the vessel in the form of a thick inner lining which, being organised and adherent, had the appearance of an intimal overgrowth. .

The above observation led me to enquire further into the pathology of coronary thrombosis, and from twenty-six cases of this condition which I was able to collect, information of a highly significant kind was obtained.*

The first case to be examined provided evidence which fully bore out the previous finding. In the circumflex branch of the left coronary artery there was a canalised thrombus about a quarter of an inch in length, with diffuse thickening of the vessel both proximal and distal to it. In fig. 1 is seen a cross section of the artery near the end of the canalised portion, where there

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* The information was obtained mainly from frozen sections, these being preferable for the study of arterial disease, not only because they are more convenient for fat staining, but also because they give a clearer representation of the variations in texture of the connective tissues than do paraffin sections. The distinction of the three layers in fig. 11, for example, would not as a rule be so well shown in a paraffin section.

are two channels, while in fig. 2 the thickened part about 4 mm. beyond this is shown, where the two channels have joined to form a single lumen. The tissue which separates the two channels in fig. 1 can safely be taken as organised thrombus, since no other pathological mechanism we know of is likely to produce such a formation, yet there is no clear line of demarcation between the new tissue and the intima and, were it not dividing the lumen of the vessel in this significant fashion, it might be taken for an overgrowth of the intima. In fig. 2 the same tissue is seen, but here, since there is one lumen instead of two channels, the picture is characteristic of atherosclerosis.

The histology of coronary thrombosis

A great variety of thrombi were found in the hearts examined, some filling the arterial lumen, others merely forming mural deposits. They varied in composition, some being pale and composed purely of fibrin, others red and consisting of masses of closely packed blood corpuscles with only a few strands of fibrin binding them together. Most were mixed, being composed mainly of fibrin but with red areas here and there. There were thrombi which appeared to be composed of platelets, forming vegetations on the walls of the arteries similar to those occurring on the heart valves, and there were also clots of gelatinous substance filling the lumina of the arteries in some regions. The thrombi were of various ages, some newly formed, others in an advanced stage of organisation, and it was from a study of the organisation that the most significant information was obtained. This can best be conveyed by the series of illustrations submitted, together with my interpretation of the process concerned.

The organisation of an arterial thrombus

When a thrombus forms in an artery it adheres to the wall, the endothelium disappears and there is an invasion of the mass by connective tissue cells from the intima. These bring about a progressive transformation of the outer layers of the thrombus into fibrous tissue, so that an advancing zone of fibrosis is formed which overruns and obliterates the original line of demarcation between thrombus and intima. The effect is an appearance of fibrous thickening similar to that seen in inflammation of the pleura or pericardium. At the same time blood vessels penetrate into the interior of the thrombus accompanied by connective tissue cells which gradually transform it into fibrous tissue. Complete organisation may thus be effected, but the progress of this change depends to a great extent on the composition of the mass. Pure fibrin, which is relatively firm in structure, is fairly readily organised, but collections of corpuscles such as are found in " red " thrombi (fig. 3) commonly undergo softening and fatty degeneration, which interfere with the process. The corpuscles break down and globules of fat appear amongst them and accumulate until they obscure everything else, so that the whole mass becomes semi-fluid or paste-like. Such masses, having no firm

ORGANISATION OF CORONARY THROMBOSIS
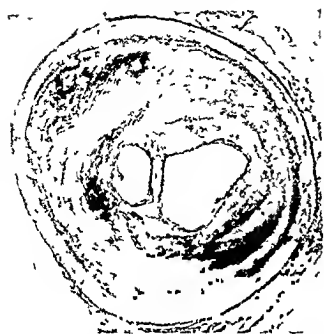
Fig 1 —Circumflex branch of left coronary artery with two channels separated by a fibrous band which represents an organised thrombus The thrombus is fused with the intima in such a way as to make the two indistinguishable The dark streaks represent fatty degeneration ×16
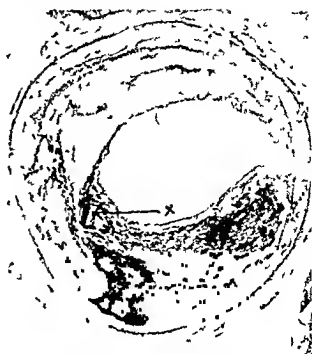
Fig 2 —Section of the same artery at a point about 4 mm distant from the section shown in fig 1, where the two channels have joined to form a single lumen The appearance is typical of atherosclerosis Fatty change is seen as in fig 1 but there is also an area of atheromatous softening at (×) ×16

Fig 3 —Recent mixed thrombus in a coronary artery The paler areas are fibrinous, the dark ones red blood clot The large black area, which is a solid mass of corpuscles, is tending to crumble and several cracks are visible in it The thrombus has become partly detached from the vessel wall, leaving a marginal space which is lined with endothelium No fat is present in the thrombus, but the black streaks in the vessel wall are old areas of fatty degeneration ×13

Fig 4 —Somewhat older thrombus, with early organisation and a marginal space lined with endothelium A layer of fibrous tissue has begun to develop in the exposed surface of the thrombus and there is organisation, with an irregular zone of fibrous encroaching on it from the intima, round the rest of its circumference Fatty change, present in considerable amount in the thrombus, is represented by the darkest patches ×13

Frozen sections Sudan III and hæmalum

ORGANISATION OF CORONARY THROMBOSIS

Fig 5—Advanced atherosclerosis with constriction of the lumen in a coronary artery The formation is essentially similar to that in fig 4, there being a mass of degenerate material encroaching on the lumen but leaving a channel at one side, a layer of fibrous tissue covers the exposed surface of the mass and a zone of organisation extends into it from the intima around its attached margins The mass is entirely fatty except for an area which is calcified at (x)   ×15
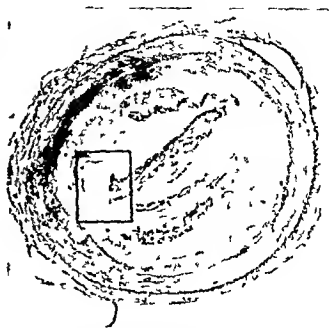
Fig 6—Recent fibrin thrombus deposited in an old thickening of the vessel wall The surface layer at its thickest part has been torn away during mounting, but part of its surface is covered by endothelium   ×15

Fig 7—Higher magnification of part of fig 6, showing the lining of endothelial cells extending from the intimal surface over the surface of the thrombus The dark patches at (x) are fibrinoid " material   ×80
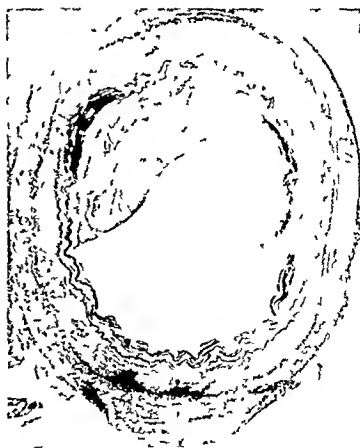
Fig 8—A fibrinous mural thrombus which is partly organised, with a sheet of fibrous tissue covering its surface There is also organisation, with an irregular formation of blood vessels invading its basal layers (see fig 9) On the rest of the intimal surface the superficial layers, which appear somewhat thickened (see explanation of fig 14), are dark stained owing to a fine fatty dusting of the tissues   ×16

Fig. 9.—Higher magnification of part of the previous illustration, showing the main mass of thrombus composed of hyaline fibrin or "fibrinoid" material (see also fig. 14) and the covering layer of fibrous tissue. The fibrinous mass is broken up and invaded by connective tissue cells, while blood vessels are penetrating it from its base. × 145.

Fig. 10.—A recent almost completely organised thrombus is seen in the left half of the artery. The fine streaks at (A) in its deeper layers are capillaries and connective tissue cells, representing organisation. The dull grey patches at (B) in the intermediate layers are unorganised fibrin; the surface layer is new fibrous tissue. The right half of the artery shows an older fibrous thickening with fatty degeneration. × 16.
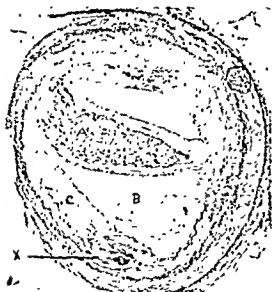
Fig. 11.—Three distinct layers of fibrous thickening are seen, one above the other. The inner layer (A) consists mostly of unorganised fibrin covered with a layer of fibrous tissue, the middle layer (B) is entirely fibrous, and the outer (C) is mostly fibrous but also contains a focus of calcification (x). × 15.
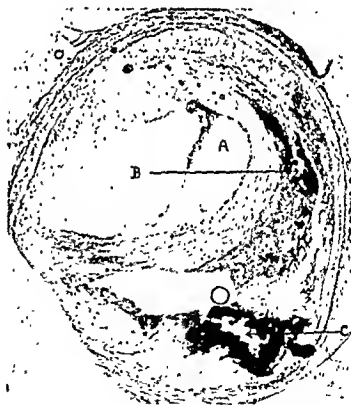
Fig. 12.—A similar formation to that shown in fig. 11 but with softening, there being three foci of atheromatous degeneration one above the other, with layers of fibrous tissue between. In (A) all that remains is the covering layer of fibrous tissue with some remnants of fatty debris clinging to it at one corner. The outer foci (B) and (C) are calcified, with the result that the section was torn in cutting. × 13.

Paraffin sections. Hæmalum and eosin

ORGANISATION OF CORONARY THROMBOSIS
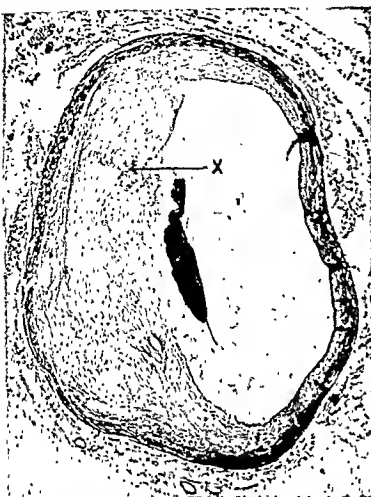
FIG. 13.—Coronary artery from a case of hypertension showing a small surface clot associated with an old fibrous thickening in which there is a patch of softening (x). There is no organisation of the clot, but separation, which occurred in embedding, has left a frayed surface. Paraffin section. Hæmalum and eosin. ×29.

FIG. 14.—The same lesion as in figs. 8 and 9, stained to show fibrin. In addition to the main mass which projects into the lumen of the vessel there are thin layers of fibrin spread over the rest of the intimal surface (x). In the frozen section (fig. 8) these layers are marked by fine fatty dusting. Paraffin section. Weigert's fibrin stain. ×55.

FIG. 15.—A higher magnification of the same lesion, showing a deposit of fibrin with endothelial cells on its surface. Paraffin section. Hæmalum and eosin. ×170.

FIG. 16.—A subendothelial streak of hyaline or "fibrinoid" material on a thickened intima. Frozen section. Sudan III and hæmalum. ×199.

structure on which the organising mechanism can build, tend to persist as areas of fatty degeneration, and it would appear as if this were one of the ways in which atheromatous patches are produced. Fatty change, it should be added, also occurs in fibrin thrombi, but in them it is not usually associated with softening and is not therefore so permanent. When the fibrin is transformed into fibrous tissue, the fats are taken up by phagocytes which tend to collect in clusters around the zones of organisation ; these form a prominent feature of atherosclerotic foci.

Canalisation

Another important feature of arterial thrombosis is seen in fig. 3. A thrombus may shrink and become partly detached from the vessel wall, leaving open spaces at its margins. Such spaces become lined with endothelium and form channels along which blood may pass. There may be several of these channels but more often there is only one, which may enlarge so that it performs more or less the function of the original lumen. The formation of the endothelial lining, which is brought about by growth of cells spreading from the free surface of the intima over the exposed surface of the thrombus, is next followed by organisation of the superficial or, as they may now be called, subendothelial layers of the thrombus, and the formation of a covering sheet of fibrous tissue over its surface (fig. 4). Progressive organisation also takes place around the adherent margins of the thrombus, so that eventually the whole mass becomes encased in new fibrous tissue, all of which is continuous with the intima of the vessel. Thus the mass now appears as if it were embedded in a thickened intima, and when fatty degeneration is present the familiar atherosclerotic nodule is produced (fig. 5).

Mural thrombi

The same process comes into play in mural thrombosis, of which there were many examples in the collection of hearts examined. Mural thrombi when recently formed are easily recognised by the fibrin or blood they contain (fig. 6) or by the signs of organisation, but when the organisation is complete these distinctive features are lost and there remains only a fibrous thickening which looks like an overgrowth of the intima.

When a thrombus forms on the wall of an artery the subjacent endothelium disappears and a new lining of endothelial cells grows over the exposed surface of the mass (fig. 7). The thrombus thus becomes subendothelial in position and organisation of its superficial layers follows, with formation of a sheet of fibrous tissue over its surface (figs. 8 and 9). At the same time organisation of its attached base also takes place and, as with the larger thrombi, it becomes encased in fibrous tissue and appears as if it were incorporated in a

thickened intima. For some time hyaline patches of unorganised fibrin may still be seen in it (figs. 7, 9, 10 and 11), and these, it would appear, account for the so-called "fibrinoid" substance which is frequently referred to in studies of atherosclerosis and to which I shall again refer later. In due course these fibrinoid masses disappear and only fibrous nodules are left.

The above sequence of changes occurs in fibrin thrombi. In "red" ones the process may be modified by softening and fatty degeneration, which again result in atheromatous foci. In frozen sections the contents of these foci tend to be washed away, leaving empty cavities such as are seen for example in fig. 12, where a red thrombus at (A) is represented simply by a band of fibrous tissue covering an empty space. The band represents the subendothelial layer of newly organised fibrous tissue while the space represents the atheromatous focus. A similar formation is seen in fig. 2 at (x). In both these sections some remnants of the softened tissue consisting of fatty globules and granular debris are seen clinging to the walls of the cavities.

Another significant feature shown in figs. 11 and 12 is the appearance of multiple layers of fibrous thickening, superimposed one on the other. In fig. 11 there are three distinct layers, the inner still containing hyaline patches of fibrin covered by the usual layer of organised fibrous tissue, while the others are almost entirely fibrous. In fig. 12 the same formation is seen, but with softening, so that there are three cavities representing three foci of atheroma one above the other, with layers of fibrous tissue between. These complex formations are commonly found in severe coronary disease and, whereas it would seem difficult to explain them on the principle of intimal overgrowth, they are not difficult to understand as the sequelæ of successively recurring mural thrombosis with organisation.

The relationship of surface deposits to atherosclerosis

Atherosclerosis is an insidious disease and the milder lesions, which we generally classify as early and which are found in the arteries of nearly all who live to middle age, can hardly be accounted for by gross thrombosis of the kind described above. We are accustomed to think of them as the result of connective tissue proliferation of a reactive type and there is evidence in some cases to support this view, but it would probably be wrong to assume that they always depend on the same process. In view of the findings described above, the possibility that some of them may represent organised deposits should be considered.

In post-mortem examinations it is not uncommon to find wisps of blood or fibrin clinging to the walls of the arteries, and since they are easily washed off they may be taken for post-mortem effects, but more careful examination will sometimes show this to be wrong. I have

mado a special search for such formations in the coronary arteries of unselected cases and found them in not a few   Fig 13 shows one from a case of hypertension in which there was comparatively little coronary disease   In the gross it looked like a small pool of blood left behind when the artery was drained but, as the section shows, it was associated with an atheromatous softening (x), so that it was presumably a genuine pathological effect   If such deposits occur at any considerable time before death they must become organised and form thickenings, unless by some process unknown to us they are dissolved   In fig 14 another form of deposit which may result in arterial thickening is seen   The section is a paraffin one, stained with methyl violet, from the same lesion as that shown in figs 9 and 10   It will be noticed that the fibrin, which stands out darkly in contrast to the fibrous tissue, is not confined to the main mass of the thrombus but extends over the surface of the intima as a series of thin films   A higher magnification of sections of part of the same vessel surface stained with hæmalum and eosin is seen in fig 15 and here a thin layer of fibrin with a rather indistinct covering of endothelial cells can be recognised   Such formations are usually found to be sub endothelial in position and this has led to the general belief that they represent changes in the intimal tissues rather than deposits on the surface   In view of the readiness with which a new layer of endothelium grows over an arterial thrombus, however, it seems doubtful if such a conclusion is justified   Hyaline streaks or patches in the intima are common, both in the subendothelial layer (fig 16) and in the deeper layers (figs 9-11), and they have attracted considerable attention in the last twenty years   Since they usually stain with methyl violet they are often referred to in the literature as " fibrinoid " and there has been considerable discussion as to their origin   Some regard them as deposits in the connective tissues and others as swellings of the tissues themselves, but, so far as I have found, only one group of writers in recent years, namely Clark, Graef and Chasis (1936), have suggested that they represent surface deposits which have subsequently been overgrown   My own observations lead me to believe that the latter is the case, and that these " fibrinoid " deposits in fact provide the essential link in the chain of evidence which connects atherosclerosis with thrombosis

DISCUSSION

That arterial thickening may be the result of thrombosis is no new idea   Von Rokitansky (1852) held that atheroma was produced by the deposition of " an endogenous product derived from the blood and for the most part from the fibrin of the arterial blood "   He noted the " metamorphosis of the deposit into a pulpy mass   consisting of a large number of crystals of cholesterin, fatty globules, and of molecules   consisting of albumin and calcareous salts " and this, no doubt, was the current view until Virchow introduced his idea of

conneetive tissue proliferation in arterial disease. Virchow (1856) dismissed Rokitansky's "encrustation hypothesis" on the ground that the deposits were subendothelial in position, and his teaching prevailed. But the encrustation hypothesis was not readily abandoned, for even 40 years later Ziegler (1896-97) in his textbook referred to fibrin thrombi in the arteries beeoming organised and forming thickenings analogous to those occurring on the endocardium. In the present century the hypothesis seems generally to have been ignored, for, except in historical notes, we seldom find reference to it in current literature.

CONCLUSIONS

In a study of coronary thrombosis evidence has been found that many of the lesions we classify as atherosclerosis are arterial thrombi which, by the ordinary process of organisation, have been transformed into fibrous thickenings. Red thrombi are prone to softening, with fatty degeneration, and many of the " atheromatous " patches found in arterial thickenings appear to be the result of this process.

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AN EXPERIMENTAL STUDY OF SOME EFFECTS OF ACUTE ANHYDRÆMIA *

G. R. CAMERON, F. BURGESS and V. TRENWITH

It is generally agreed that the initial stages of a thermal burn are dominated by excessive fluid loss at the site of burning and the acute anhydræmia which accompanies it. Uncertainty still exists about the later features, especially the disturbance of nitrogen metabolism and the anæmia which so often develop. To simplify the problem, we have studied some of the effects of rapidly progressive anhydræmia produced in such a way as to eliminate local tissue damage and the tendency to infection, complications which obscure the late burn picture. Davis (1940 a and b, 1941) has also employed the same method for pathological studies of burn dehydration and Stoner and Green (1945) for the investigation of the adenosine equivalent of the blood in dehydration shock. Marriott (1923) has reviewed the early literature on anhydræmia.

Methods

Rabbits and a few non-pregnant female goats were injected subcutaneously with large amounts of sterile hypertonic glucose (60 per cent.) and sodium chloride (20·25 per cent.) solutions. The two hind limbs were chosen as sites of injection and precautions against infection were maintained. It was sometimes necessary to employ anæsthesia, especially with saline injections, and a light dose of Nembutal was used so that recovery might be rapid. Blood investigations were carried out thereafter at regular intervals, using methods described in a previous paper (Cameron et al., 1945). Animals were killed for pathological examination 6, 24 and 48 hours after injection. For the study of urinary N excretion rabbits were kept in metabolism cages except when being fed. They were catheterised at the commencement of the experimental period but not subsequently. Unlimited green food and oats were available for each animal but no water was given. Blood pressures were measured with the Grant and Rothschild (1934) apparatus in a quiet warm room.

Results

1. Goats tolerate large doses of 60 per cent. glucose and as much as 20 c.c. per kg. can be injected without fatal results. Most rabbits recover from similar doses, though invariably a few die during the first 24 hours after injection. Table I summarises the rabbit experi-

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* A report to the Burns Subcommittee of the Medical Research Council's War Wounds Committee.

ments, table II the goat experiments. These indicate much the same sort of results in both species. Within a few hours of injecting hypertonic glucose the hæmoglobin percentage (Haldane scale), red cell count and packed cell volume (bæmatocrit) begin to rise and steadily increase to a peak somewhere between 12 and 20 hours in the goat, probably earlier in the rabbit. The plasma volume falls throughout this period as œdema at the site of injection develops. Serum protein concentration after a slight initial rise usually remains steady, whilst blood non-protein-nitrogen, especially urea, increases. Goats show an increased circulating blood cell mass as judged by calculations from the circulating plasma volume and the hæmatocrit percentage. Generally speaking, these disturbances have subsided by 48 hours after injection. They' are most simply interpreted as signs of massive fluid loss from the circulation due to an osmotic action of the hypertonic solution injected subcutaneously. Very little protein is lost from the blood, since the serum protein concentration is unchanged, hence there can have been no great alteration in capillary permeability for proteins and no capillary damage. The condition is one of uncomplicated acute anhydræmia which passes off within 48 hours of onset, presumably through compensatory withdrawal of fluid from various tissues.

2. Accompanying these blood changes are well defined clinical features. The respiratory rate increases, the pulse rate becomes very rapid and the rabbit is huddled up with a drooping head. It apparently feels cold and after some hours of progressive anhydræmia becomes weak and loses the power of movement in the hind limbs. Intermittent muscular spasms may develop, associated with teeth-grinding, great thirst and anorexia. The animal is difficult to bleed because of collapsed veins. At this stage, convulsions may set in, leading to death in a short time, but more often the condition slowly improves, though there may be relapses. Goats behave in much the same way. The blood pressure falls steadily in rabbits from a normal figure of 85-90 mm. Hg to 40-60 at 6 hours and 35-45 at 24 hours, the fall being most pronounced in animals showing severe œdema and alarming clinical features. There seems little doubt that progressive circulatory collapse parallels the loss of fluid from the blood and may be responsible for a fatal termination.

3. Pathological changes throughout this period are not striking. They chiefly affect the circulatory system. Œdema sets in quite soon at the site of injection of hypertonic glucose and rapidly increases throughout the cutis, subcutaneous tissues and near-by muscle. It may spread well beyond the limbs to the lumbar region but it is never generalised throughout the body. It is gelatinous, often loculated and firm. Staining with a little blood is not uncommon. The affected muscle becomes pale but necrosis does not follow. Occasionally a few small hæmorrhages are found in the muscle and at the margins of the œdematous area, where the veins too may be congested. Œdema

reaches its greatest development about 24 hours after injection and appears to remain stationary in the next few days. Only rarely does it become infected.

Six hours after the onset there is widespread congestion of vessels throughout the body, with dehydration. The heart may show epicardial and endocardial petechiæ ; a cyanotic hue and some degree of dilatation are seen in the rare cases which are fatal at this stage. There is no pericardial fluid and the heart muscle feels dry. The lungs are often uniformly congested, show subpleural petechiæ and sometimes large hæmorrhages, and feel dry. Pleural fluid is barely recognisable. The liver is not enlarged, though it seems congested and may be plum-coloured. Gall bladder and bile ducts present no consistent changes. The pancreas is dry and diffusely congested. The spleen is often smaller than normal, cyanotic, firm and poor in blood. Subcapsular hæmorrhages are rare. The kidneys are usually cyanotic and congested, oozing dark-coloured blood when cut, but the surface may be dry and sticky. Ureters are congested. The bladder contains a small amount of urine. It is common for the animal to pass a large amount of urine soon after being injected with hypertonic glucose. The peritoneum is frequently congested and may show petechiæ. Peritoneal fluid is scanty or absent. Congestion and petechiæ are scattered throughout the mucous coat and serosa of the stomach and small intestine. Fæces are normal. No constant change has been noted in the ductless glands, lymph nodes, bone marrow or central nervous system.

At 24 hours, all these features are more pronounced and congestion and tiny hæmorrhage formation may be severe in the cardiovascular system, lungs, liver and kidneys. The rabbit's stomach not infrequently shows numerous tiny hæmorrhagic erosions and even small mucosal ulcers. The tissues everywhere feel dry and the subcutaneous tissue may have a shrunken appearance. By 48 hours, most of these features have passed off and the hæmorrhages are fading.

Microscopical examination adds very little to the naked-eye findings. There is no evidence of serious damage at the site of introduction of the fluid. Muscle fibres retain their normal detail. Œdema fluid infiltrates the fibre spaces. No pronounced leucocytic infiltration is apparent. We have found no lesions in the main organs other than capillary and venous congestion, inconstant petechiæ and on a few occasions small areas of pulmonary œdema. Careful examination of the liver, kidneys, adrenals and heart failed to convince us of the presence of parenchymatous damage.

The main changes following upon acute anhydræmia are thus widespread capillary and venous congestion, inconstant petechiæ in a number of organs and serous membranes, contraction of the spleen and dehydration of tissues. The circulatory changes are similar to those found in other conditions with a diminished blood volume, *e.g.* hæmorrhage, and are probably referable to constriction of peri-

pheral arterioles which leads to blood stasis in the smaller peripheral vessels (Marriott).

4. Tables I and II give information about the blood non-protein nitrogen in anhydræmic rabbits and goats and some data on urinary urea excretion during the 11–14 days after the onset of the condition.

TABLE I

Effects of injecting rabbits subcutaneously with 20 c.c. per kg. of 60 per cent. glucose solution

Time interval	Hb (per cent.)	R.B.C. (millions per c.mm.)	Serum protein (g. per cent.)	Blood N.P.N. (mg. per cent.)	Total urine in 24 hours (c.c.)	Total urinary urea excreted in 24 hours (g.)
Expt. 1. Means of 8 rabbits						
24 hrs. before injection	77	5·8	7·3	46	...	...
Glucose injected subcutaneously						
After 5 hrs.	108	7·8	8·0	57	...	...
,, 24 ,,	98	7·2	7·4	93	...	...
,, 2 days	78	6·2	...	...	...	...
,, 3 ,,	68	5·5	7·1	79	...	...
,, 4 ,,	60	5·0	7·6	68	...	...
,, 5 ,,	58	4·8	7·5	47	...	...
,, 6 ,,	63	5·2	...	...	...	...
,, 7 ,,	72	4·9	7·6	40	...	...
Expt. 2. Means of 5 rabbits						
24 hrs. before injection	89	...	...	44	56	1·76
Glucose injected subcutaneously						
After 5 hrs.	121	...	...	78	...	...
,, 24 ,,	109	...	...	175	90	1·33
,, 2 days	78	...	...	141	65	1·61
,, 3 ,,	75	...	...	84	63	2·13
,, 4 ,,	74	...	...	61	55	2·27
,, 5 ,,	75	...	...	42	37	1·85
,, 7 ,,	78	...	...	60	48	2·71
,, 8 ,,	81	...	...	42	96	2·48
,, 11 ,,	86	...	...	...	...	...

An increase in N.P.N. commenced in rabbits within 5 hours and was maintained for about 3 days. The peak occurred about the 24th hour and was associated with a slight fall in total urinary excretion of urea despite the urinary output being somewhat increased. It could not be accounted for by concentration of the blood constituents. A small amount of albumin appeared in the urine at this time but

TABLE II—*Effects of injecting goats subcutaneously with 20 c.c. per kg of 60 per cent glucose solution*

Time interval	Hb (per cent)	R B C (millions per c mm)	Hæmatocrit (per cent)	Blood volume (c c) Total	Plasma	Cell	Total serum proteins (g per cent)	Blood N P N (mg per cent)
Goat 22, ♀ Body weight 33 9 kg								
Immediately before glucose	76	10 27	20 7	2620	1020	700	6 58	27
Glucose subcutaneously								
After 4 hrs	94	22 67	33 4	2390	1595	705	6 73	27
„ 8 „	102	24 40	35 0	2330	1490	840	7 14	30
„ 12 „	111	27 30	39 8	2080	1250	830	6 55	30
„ 16 „	112	27 82	42 7	2050	1176	875	6 83	38
„ 20 „	116	20 37	42 0	2060	1175	885	0 83	38
„ 24 „	100	27 25	41 3	2260	1325	035	0 50	50
„ 2 days	04	25 12	34 8	2450	1620	850	6 61	46
„ 3 „	78	19 46	28 0	2800	1875	725	5 85	23
„ 4 „	74	18 76	28 2	2510	1800	710	5 46	23
„ 8 „	68	17 00	27 0	2510	1875	095	5 44	23
„ 8 „	65	16 82	25 4	2420	1800	620	5 52	23
„ 10 „	58	16 01	23 6	2490	1900	590	7 02	28
„ 13 „	63	16 70	25 1	2540	1900	040	6 74	28
„ 15 „	68	16 75						
„ 20 „	84	16 90						
Goat 23, ♀ Body weight 32 6 kg								
Immediately before glucose	78	15 97	28 8	2530	1800	730	7 24	46
Glucose subcutaneously								
After 4 hrs	90	17 47	32 8	2540	1710	830	6 90	46
„ 8 „	97	17 47	30 3	2270	1445	825	7 52	40
„ 12 „	111	21 47	40 7	2390	1410	980	7 82	49
„ 16 „	117	23 50	42 0	2340	1360	080	7 82	40
„ 20 „	113	23 37	41 4	2005	1175	830	7 45	57
„ 24 „	101	21 37	39 0	2110	1290	820	7 43	100
„ 2 days	86	10 87	33 0	2420	1600	820	6 37	68
„ 3 „	82	16 42	29 4	2420	1710	710	6 34	40
„ 4 „	73	15 00	28 7	2470	1760	710	6 35	39
„ 0 „	65	13 97	27 6	2500	1810	090	6 35	39
„ 8 „	65	14 21	26 0	2510	1860	650	7 36	35
„ 10 „	61	13 56	24 1	2610	1900	610	6 85	34
„ 13 „	60	13 76	23 7	2520	1920	800	7 71	32
„ 15 „	00	12 06						
„ 20 „	66	14 23						
Goat 24, ♀ Body weight 36 6 kg								
Immediately before glucose	08	13 87	23 7	2650	2020	030	7 18	20
Glucose subcutaneously								
After 4 hrs	73	20 87	27 0	2520	1840	670	7 96	22
„ 8 „	88	21 12	32 6	2540	1710	830	7 07	20
„ 12 „	104	24 62	34 5	2290	1470	780	7 04	25
„ 15 „	104	24 87	40 0	2060	1220	840	7 14	38
„ 20 „	100	21 87	40 2	2040	1220	820	6 55	52
„ 24 „	100	21 12	35 8	2290	1470	820	0 19	62
„ 2 days	88	19 87	32 7	2470	1650	820	5 90	30
„ 3 „	71	14 62	25 2	2570	1920	650	6 20	10
„ 4 „	70	14 25	24 2	2510	1900	610	6 20	18
„ 0 „	64	13 39	23 0	2520	1920	600	0 20	18
„ 8 „	63	13 06	22 4	2470	1920	550	8 48	18
„ 10 „	55	12 53	20 0	2380	1900	480	6 55	20
„ 13 „	55	12 05	21 0	2430	1920	610	7 20	23
„ 16 „	55	12 36						
„ 20 „	54	12 25						

soon disappeared. There may have been a temporary disturbance of renal function, though it seemed far from impressive. No histological changes were found in the kidneys. During this upset, the animals were ill and took very little food. Possibly because of this, endogenous metabolism was increased. The goats, too, showed a rise in their N.P.N. values but this was much less pronounced and shorter in duration than with rabbits. On the other hand, the goats were not so ill as the rabbits. Urinary excretion could not be followed in the goats because of technical difficulties. We conclude from these experiments that acute anhydræmia is associated with a temporary disturbance of nitrogen metabolism which is not attributable to any gross upset in renal function.

5. Tables I and II also show that anæmia develops in anhydræmic animals, both the hæmoglobin and red cell values falling, the former much more than the latter. In all three goats the packed cell volumes decreased simultaneously; since the plasma volume during this time kept more or less steady around normal values it would seem that a decrease in circulating blood cell mass had occurred, but whether this was the result of destruction of cells or their being side-tracked into stagnant pools of the circulation we cannot say. There was no evidence that the anæmia was due to blood dilution from increased intake of water, though it is known that increasing the water supply after a period of dehydration causes an immediate dilution of the blood and an abrupt fall in hæmoglobin concentration (Underhill and Kapsinow, 1922). We conclude that anhydræmia alone can bring about temporary anæmia some days after recovery from the acute phase.

6. Closely similar results follow the use of hypertonic sodium chloride solutions.

Discussion

In an attempt to simplify the problems afforded by a thermal burn we have studied the effects produced by acute anhydræmia after the subcutaneous introduction of hypertonic solutions of glucose and sodium chloride. This form of anhydræmia has the advantage of not being complicated by the presence of necrotic tissue, as with a burn, nor is it associated with the action of a toxic agent or grossly abnormal physical conditions, as in most previous experiments (cf. Marriott). It differs from the anhydræmia of burns in consisting of a loss mainly of the watery portion of the blood, the protein remaining unaffected. Nevertheless they have in common a stage of hæmoconcentration from decrease in circulating plasma volume and the pathological picture presents many points of resemblance (compare for instance the descriptions given by Pack, 1926; Colebrook et al., 1944; and Cameron et al., 1945, for thermal burns), including a tendency towards disturbance of nitrogen metabolism and delayed transient anæmia. A degree of hæmoconcentration and anhydræmia

similar to that with severe burning can be evoked simply by varying the dose of hypertonic fluid, and when this is done the resemblance between many of the features typical of burns and those of simple anhydræmia is indeed close. It is difficult to escape from the conclusion that such features of burns are the result of anhydræmia alone and that there is no need to postulate any other factor than loss of fluid from the blood. This view, supported by Underhill and his collaborators and others (Underhill, Carrington, Kapsinow and Pack, 1923; Underhill, 1927, 1930; Underhill, Kapsinow and Fisk, 1930; Blalock, 1931; Harkins, 1935, 1938; Colebrook *et al.*, 1944), does not deny the importance of complications such as infection, blood destruction by thermal or other action and tissue necrosis in contributing to the grave systemic disturbances which follow burning. It is our desire to emphasise the importance of a measurable and concrete process such as acute fluid loss from the circulation in explaining, at any rate in part, the pathology of severe burning. Until more convincing evidence of the action of burn toxins is produced, we feel that the anhydræmia theory is justified as a working hypothesis.

It is not sufficient, however, in an analysis of burn pathology to rest content with such an attitude. The main problem must be carried a stage further and the question propounded, why does anhydræmia produce such severe effects? Davis (1941), who has obtained much the same results in his careful study of dehydration in dogs injected with hypertonic saline, emphasises the importance of anoxæmia in such cases. The pathological picture is certainly reminiscent of that described by Yant *et al.* (1934), who exposed dogs to atmospheres containing very low oxygen concentrations, and Campbell (1927), who studied chronic anoxæmia.

Diminished blood volume leads to compensatory constriction of the peripheral arterioles and a greatly decreased volume flow of blood through certain parts of the body (Marriott), especially the extremities. It is not known how far this affects blood flow through the internal organs. Cardiac function is said to be upset in anhydræmic infants (McCulloch, 1920). Blood pressure is often well maintained, though it falls when plasma loss becomes excessive. These observations are highly suggestive of cardio-vascular functional disturbance and at least imply that oxygen supply to the tissues may be impaired. But we cannot assume such a state of affairs until break-down of compensatory mechanisms has been proved. For a decision, there must be resort to further experimentation and an investigation of tissue respiration is urgently required.

Summary

1. Subcutaneous injection of large amounts of hypertonic glucose or sodium chloride solutions produces severe local œdema, acute anhydræmia, hæmoconcentration and circulatory collapse.

2. A temporary disturbance of N metabolism, indicated by a rise of blood non-protein nitrogen in the absence of pronounced renal failure, and a delayed transient anæmia may follow on such conditions.

3. Pathological changes resemble those associated with severe thermal burning.

4. It is reasserted that many of the disturbances accompanying burning are the result of acute anhydræmia alone.

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THE ABO BLOOD GROUPS IN THE UNITED KINGDOM; FREQUENCIES BASED ON A VERY LARGE SAMPLE

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DURING the European war this Unit has had the opportunity of diagnosing the ABO blood groups of large numbers of people, mainly representing a mixed population of the United Kingdom, but the blood of smaller numbers of persons from Continental Europe was also examined. This very big undertaking was organised by the late Dr G. L Taylor in co-operation with medical officers of the Royal Air Force.

TECHNIQUE

In all the tests the technique described in a paper in this *Journal* (Taylor *et al*, 1942*a*) has been used This method employs both the testing of the red blood cells for iso agglutinogens and the determination of the iso agglutinin content of the serum, all tests being performed in precipitin tubes and at room temperature

No diagnosis has been included in these compilations in which a sample of serum as well as of red cells was not examined. In all but nine cases where the diagnosis from the red-cell reaction was that of group B, the serum of the person concerned was also tested with group A_2 cells ; the positive reaction which resulted thus confirmed the fact that the blood examined could not have been of group A_2B instead of group B. Insufficiency of serum prevented this test with A_2 cells in the nine cases mentioned, but to avoid a selective effect on the total figures these results were included on the basis of their red-cell reactions

The χ^2 value for each batch of results has been calculated by a simple method suggested by Professor Fisher, in which the observed values in the O, A and B groups are used to calculate the expected value of the AB group, which, in turn, is used to evaluate χ^2.

As an example of the method used, the calculations for the Belgian sample are shown below. To each number has been assigned an arbitrary letter of tho alphabet and by this means the steps in the calculation of χ^2 are explained. We are not competent to give any theoretical explanation, merely a recipe for fellow non mathematicians

The gene frequencies are readily obtained from the quantities s, t, u and v by the use of the simple formulæ —

$$O = \frac{s}{v}, \; A = \frac{t-s}{v} ; \; B = \frac{u-s}{v}.$$

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* Working on behalf of the Medical Research Council.

Belgian sample

Number in group		Square root of number	
O	142	11·916375	$s = \sqrt{O}$
O+A	302	17·378147	$t = \sqrt{O+A}$
O+B	175	13·228757	$u = \sqrt{O+B}$
		18·690529	$v = t+u-s$
	349·33587	$w = v^2$	
O+A+B	335		
	14·33587	$x = w-(O+A+B)$ = expected number of AB	
AB	17	y	
	−2·66413	z = deviation = AB (expected −observed)	
		$= y - x$	

$$\text{Variance} = \frac{wx}{tu}$$

$$\chi_1^2 = \frac{(\text{deviation})^2}{\text{variance}} = \frac{tuz^2}{wx} = 0\cdot3258 \text{ for 1 D.F.}$$

RESULTS (TABLE I)

European samples. The numbers in many cases are not large, but are put on record as having been obtained by the examination of both cells and serum. They are probably the most recent figures available for those peoples. The men tested were airmen; the figures for their national groups must be taken, therefore, as averages for their countries since we have no information about their districts of origin. Some comparisons with data obtained by other workers and quoted by Boyd (1939) may be of interest.

The Belgian figures given in our paper differ rather markedly from those obtained by Moureau in Liége, whose results in percentages were 46·7 O, 41·9 A, 8·3 B and 3·1 AB.

No Czechoslovakian figures exist with which our findings can be compared, as the group of men we examined would contain Czechs and Slovaks in unknown proportions.

Our Dutch results are in very close agreement with those obtained by van Herwerden and Boele-Nijland when examining students (45·7 O, 41·2 A, 9·6 B and 3·5 AB). Both a student population and the airmen whose blood we tested would tend to be heterogeneous as to place of origin.

Probably the figures obtained on French soldiers during the War of 1914-18 by the Hirschfelds would be the most suitable for comparison with our data. The chief difference here is that they found a greater frequency of group B (43·2 O, 42·6 A, 11·2 B and 3·0 AB).

Boyd gives the results of many investigators in various parts of Poland, and by inspection of his list it can be seen that our figures lie within the wide range of group frequencies there given.

We tested only 198 Turks; the results from this relatively small

TABLE I

Results of ABO blood group tests done by the Galton Laboratory Serum Unit, 1939–1945

Nationality	Number observed					Phenotypic frequencies				Gene frequencies			χ^2	Approximate probability
	O	A	B	AB	Total	O	A	B	AB	O	A	B		
Belgian	142	160	33	17	352	40 341	45 454	9 375	4 830	63 750	29 222	7 022	0 325	0 58
Czechoslovakian	56	91	31	19	197	28 426	46 193	15 735	9 015	53 573	33 225	13 202	0 120	0 73
Dutch	253	233	52	18	556	45 504	41 907	9 353	3 237	67 388	26 010	6 502	0 046	0 84
French	1234	1107	263	80	2780	44 389	43 058	9 460	3 094	66 145	26 816	6 739	1 524	0 22
Polish	207	250	110	55	631	32 805	39 620	18 859	8 710	57 445	27 000	14 645	0 100	0 60
Turkish	64	07	25	22	108	37 273	48 990	12 620	11 111	53 142	35 723	11 136	1 730	0 19
Cambridge—Males	1015	1817	351	125	4338	45 894	42 874	8 282	2 050	67 604	26 451	5 855	0 220	0 05
Females	2101	2084	412	140	4806	44 965	43 303	8 673	3 100	66 091	26 901	6 108	0 369	0 50
Total	4106	3901	703	274	9014	45 400	43 134	8 437	3 030	67 122	25 690	5 989	0 580	0 45
R.A.F.	88,782	79,334	15,280	5781	190,177	46 684	41 716	8 560	3 040	68 310	25 690	6 000	0 839	0 38
R.A.F.+Cambridge	92,888	83,235	17,043	6055	199,221	46 626	41 780	8 655	3 039	68 206	25 735	5 999	1 123	0 29
(University College, London*)	1503	1640	297	113	3459	43 452	44 695	8 585	3 267	65 848	27 939	0 213	0 326	(0 58)

* Ikin et al (1939)

number are not in agreement with those of other workers as quoted by Boyd; nowhere was such a high frequency of group AB found as in the bloods examined by us. Previous workers, if testing only the red cells, may well have classed some weak A_2B persons as group B, especially if the testing sera were not very strong; this might well be the explanation of the discrepancy in results, as the more reliable data all show a higher frequency of group B than was found by us.

British samples. The figures for the United Kingdom (*i.e.* R.A.F. plus Cambridge civilians) given in the present paper should be considered as replacing those published in our earlier paper (Taylor *et al.,* 1942*a*), as a portion of those results are incorporated in the larger numbers here.

Cambridge civilians. The figures for Cambridge civilian blood donors do not represent a typical East Anglian population, since a large proportion of these people were university undergraduates and came from all parts of the United Kingdom. In passing from Southern England towards the North-West and towards Scotland the frequency of group O increases and that of group A simultaneously decreases (Fisher and Taylor, 1940; Fraser Roberts, 1942). If the Cambridge civilian data are divided according to sex it is interesting to find that the males show a slightly higher incidence of O and a lower incidence of A than the females. This might be foreseen, since a far greater proportion of the women tested were local residents, the number of Girton and Newnham students available being much smaller than the number of students from the men's colleges.

R.A.F. sample. The figures from this large sample also indicate an average distribution corresponding to the Midlands, and differ markedly from those reported from University College London for a predominantly Metropolitan population (Ikin *et al.,* 1939), which are included in table I for comparison.

Anomalies in results

Apart from anomalies due to errors in recording and those caused by technical mistakes in distributing reagents to the tubes, both of which were rectified by repeating the original tests on the samples concerned, several classes of unexpected results may occasionally occur and necessitate further tests to elucidate their cause.

(*a*) *False positive reactions.* These sometimes occurred with the cells under examination and sometimes with the serum under test with the known A_1 and B cells. The former were almost always due to the cell suspensions being in poor condition and a re-test with a fresh suspension of cells obtained from the clot of the whole blood specimen usually gave a definite result. In cases where the re-test was still unsatisfactory a fresh sample of blood was requested and no result was scored unless this was obtained. In a few cases the

persisting reactions were due to cold agglutinins, to which reference is made in paragraph (b). In one case—an undoubted group A according to its serum reactions—the red cells reacted normally with the anti-A serum but also gave small but definite reactions with several anti-B sera and with the sera of two out of three AB bloods. Repetition of the test at 37° C. produced a small reaction with one anti-B serum but complete lysis in all the other tubes. These anomalous results persisted when two further samples of this man's blood were examined and we regret that circumstances did not permit a fuller investigation of the agglutinogen responsible. When the serum gave unexpected positive reactions with the A_1 or B cells, further tests were done, their nature depending on the blood group of the sample concerned, as given by the red-cell reactions. These are detailed in sections (b) and (d).

(b) *Cold agglutinins.* Occasionally, anomalous positive reactions which persisted on re-testing at room temperature were found with either the cells or serum under examination and in two instances with both cells and serum of the same group B persons. The grouping test was then repeated at 37° C.; in all cases the anomalous reactions disappeared, leaving only the appropriate iso-agglutination reactions, and cold agglutinins were assumed to be responsible. In the whole British series examined we found sixty-one such cases.

(c) *Missing agglutinins.* Agglutinins are often absent in infancy, but the absence of an expected agglutinin in an adult is very rare; we found only sixteen examples in the 199,221 British cases reported in this paper. Fifteen of these concerned the absence of a reaction expected with B cells, three in group O and twelve in group A bloods, due to a deficiency or perhaps a complete absence of anti-B agglutinin. There were many more in which the reaction, though present, amounted merely to a " w " (weak) on our scale of notation.

Only one case—that of a group O person—occurred in which there was a complete absence of anti-A, although the anti-B agglutinin was present in normal amount (++ reaction with group B cells). In all such cases the cells and serum of the person concerned were re-tested and, if the expected agglutinin was not found, the group diagnosis was made on the reaction given by the red cells.

In one instance the serum of a group O person was apparently very deficient in anti-A, but titration with A_1 cells showed that the weak reaction in the routine grouping test was due to a " zone " in the titration at low dilutions of serum, and the serum had, in fact, a titre of 256 with A_1 cells.

(d) *α_1 occurring in sera of groups A and AB.* When the serum of a group A or AB person gave an unexpected positive reaction on testing with A_1 cells, the test was repeated, using in addition known A_2 and O cells and putting up a duplicate set in the 37° C. incubator. Almost always the agglutinin responsible for the original reaction with the A_1 cells was α_1; on re-testing no reaction would be found with

either the A_2 or O cells, even if it did still occur with the A_1 cells, and no reaction with the A_1 cells could be demonstrated at 37° C.

In a paper in this *Journal* (Taylor *et al.*, 1942*b*), estimates were made of the frequency of occurrence of the irregular iso-agglutinin α_1 ·in the sera of persons of the sub-groups A_2 and A_2B. It is now possible to extend this investigation to the much larger series reported in the present paper. For this purpose only the 199,221 persons included in the R.A.F. and Cambridge civilian data have been considered, since the ratio $\dfrac{A_2}{A_1+A_2}$ is not necessarily the same for the other national groups. Using the value of 21·9524 per cent. found by us (Ikin *et al.*) for the ratio $\dfrac{A_2}{A_1+A_2}$ in England, we can estimate the numbers in the sub-groups A_2 and A_2B among the 83,235 group A and 6055 group AB persons tested. The results can be seen in table II.

TABLE II

Occurrence of α_1 in sera of groups A and AB

Group	Number in group	Estimated number in A_2 sub-group	Number containing α_1 on first test				Percentage of sub-group showing α_1
			On second test		Not re-tested	Total	
			Present	Absent			
A	83,235	18,272	169	226	12	407	2·2
AB	6055	1329	257	63	29	349	26·3

The reactions given by the α_1 in the sera of the sub-groups A_2 and A_2B tend to be weak and, as will be seen from the table, positive reactions were not found on re-test in all cases. Failure to repeat the positive findings might have been due to a weakening or disappearance of the α_1, as the second tests were usually done a day after the first and antibody in small amounts is very labile. On the other hand, those reactions which were not repeatable might not have been true reactions. The cases where no re-tests were done occurred in the early part of the R.A.F. work; at that time we assumed that a small reaction with A_1 cells by the serum of a group A or AB was due to α_1, as it almost certainly must have been. The figures, therefore, for the percentages of the sub-groups containing α_1 in demonstrable concentrations (being calculated on the first test totals) are probably rather too high, but they would appear to indicate at least the order of frequency of occurrence of α_1 in the sera of A_2 and A_2B persons.

Among the 257 group AB persons whose sera contained α_1, confirmed by the second test, were two in which the antibody was present in such concentration that the serum could be used as an α_1 serum for the sub-grouping of A and AB bloods.

(e) *Hæmolysis of A cells in the agglutinin test.* It is perhaps worth noting that, occasionally, hæmolysis of the A_1 cells occurred when the serum of a group O person was being tested. When this happened the serum concerned was heated at 56° C. for ten minutes and the test repeated; no hæmolysis ever occurred in the second test when this heated serum was used.

SUMMARY

The ABO blood groups of a very large number of unrelated people have been determined by tests on both red cells and serum. Data are given for a large number of persons from the United Kingdom, and also for smaller numbers of men of Belgian, Czech, Dutch, French, Polish and Turkish nationalities. The following distribution of groups was found in 190,177 members of the Royal Air Force: O 46·684 per cent., A 41·716 per cent., B 8·560 per cent., and AB 3·040 per cent. These figures may be considered to be representative of Great Britain as a whole.

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THE COLLAGENASE (κ TOXIN) OF
CL. WELCHII TYPE A

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IN clinical gas gangrene due to infection with *Cl. welchii* one of the most striking features is the conversion of large amounts of muscle to a soft friable pulp. In 1923 Henry claimed that pieces of live rabbit muscle incubated with *Cl. welchii* filtrates swelled up, became opaque and underwent changes similar to those occurring in gas gangrene ; in the process it absorbed much of the lethal factor in the filtrates without producing much effect on the hæmolytic factor. No quantitative experiments were made, and the qualitative results were used only to support the view that the hæmolytic and lethal factors in *Cl. welchii* filtrates are distinct.

Though these remarkable observations have been much quoted, no one seems to have repeated them until Macfarlane and MacLennan (1945) showed that live human or rabbit muscle incubated with *Cl. welchii* type A filtrates fell to pieces, the pieces being unaltered muscle fibres deprived of their reticulin scaffolding (Robb-Smith, 1945). They regard the active substance as a collagenase * and point out its possible importance in the spread of gas gangrene.

Though the muscle-disintegrating activity of the *Cl. welchii* filtrates used by Macfarlane and MacLennan is neutralised by *Cl. welchii* type A antisera, no evidence is yet available to determine whether this activity is due to any of the toxins of *Cl. welchii* so far described or whether more than one substance is responsible for it. We have tried to tackle these problems from the immunological standpoint.

Experiments with muscle

We have found that pieces of fresh guinea-pig muscle incubated overnight with *Cl. welchii* type A filtrates squash far more readily under finger pressure than similar pieces incubated with broth and that this muscle-softening activity is neutralised by *Cl. welchii* type A antisera. Muscle can therefore be used as an indicator to show whether this effect is due to one substance only and whether it is or

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* Maschmann (1938) claimed that *Cl. welchii* filtrates contain a collagenase but later (1938-39) he seemed less certain.

is not due to one or other of the recognised toxic substances in *Cl. welchii* type A filtrates. For these purposes we took one of our sera (R 8531) as standard, ascribed to it an arbitrary value of 180 " units " and tested a number of other sera against it for their capacity to inhibit softening of guinea-pig muscle by several different *Cl. welchii* type A filtrates produced in very different media.

Method. In a series of Lambeth tubes run out the test dose of the filtrate under test (usually at 2 units), amounts of serum differing by 10 per cent., and sufficient saline to make up to constant volume. After the mixtures have stood for half an hour add to each a piece of muscle (*ca.* 6 mm. square) from the abdominal wall of a freshly killed guinea-pig, cork the tubes and incubate at 37° C. in a water-bath overnight. In the morning remove the pieces of muscle to the squares of a chequered tray and prod each with the finger to determine whether softening has occurred. The end-point is taken as the tube which contains the maximum amount of antiserum allowing softening to occur.

Table I shows the kind of result obtained. It is clear that, for these six filtrates at least, the values obtained for each serum agree

TABLE I

Comparison of anti-muscle-softening values of sera determined against six different Cl. welchii *filtrates*

Serum	Serum values against *Cl. welchii* filtrate					
	AG 835	AG 886	AG 893	AG 938	AG 952	AG 956
R 8531	180	180	180	180	180	180
R 5434	50	50	50	50	50	70
A 16836	110	100	130	130	95	130
GGC 3296	500	450	550	530	600	600
LX 251	800	650	900	700	700	...
H 3044	500	500	500	500	600	500
A 24422	180	160	140	150	130	130
R 7843	330	300	320	300	340	300
R 5603	900	870	650	700	900	...
R 7577	70	65	90	70	80	80
H 9564	...	210	280	210	210	230
Ex 770	...	400	...	330	350	320
H 2917	...	700	800	700	800	...
Test dose (α)	0·32 ml.≡ 1α unit	0·37 ml.≡ 1α unit	0·4 ml.≡ 1α unit	0·26 ml.≡ 1α unit	0·26 ml.≡ 1α unit	0·26 ml.≡ 1α unit
Test dose (muscle-softening factor)	0·9 ml.≡ 2 units	0·83 ml.≡ 2 units	0·9 ml.≡ 2 units	1 ml.≡ 2 units	0·6 ml.≡ 2 units	0·95 ml.≡ 2 units

very well and it is therefore unlikely that more than one muscle-softening substance is present in these filtrates.*

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* When fresh guinea-pig muscle is incubated overnight with some *Cl. welchii* type A filtrates—especially those prepared by growing the organism in peptic digests of horse muscle—a slimy change is produced independent of softening and easily distinguishable from it.

If the results obtained by titration of sera against the muscle-softening factor are now compared with the known anti-α, anti-θ and anti-hyaluronidase values of these sera (table II) it is clear that these

TABLE II

Comparison of anti-muscle-softening values of sera with their anti-α, anti-θ and anti-hyaluronidase values. The anti-muscle-softening value used is the average value against several different filtrates

Serum	Anti-α value	Anti-θ value	Anti-hyaluronidase value	Anti-muscle-softening value
R 8531	170	90	640	180
R 5434	0·2	100	320	55
A 24422	27	13·5	<20	150
A 16836	57	500	<20	110
R 6423	75	70	320	<2
H 2017	100	900	320	750
R 7843	140	130	<20	320
H 9564	270	360	320	230
R 5603	280	0·7	640	800
GGC 3296	370	38	1280	540
R 7577	420	11	<5	77
LX 251	460	0·2	640	700
H 3044	520	62	320	500
Ex 770	620	105	120	360

sera do not neutralise the muscle-softening factor in proportion to their anti-α, anti-θ or anti-hyaluronidase values. It is therefore unlikely that the muscle-softening substance is either α toxin, θ toxin or hyaluronidase : it is probably a new toxin, which we propose to call κ (kappa) toxin.

Experiments with collagen

Though testing with pieces of muscle was easy enough—to our great surprise testing to about 10 per cent. offered little difficulty—it was of little use for large scale work. So, as we knew from Robb-Smith's work that disintegration of muscle was associated with the destruction of reticulin, we tried collagen as an indicator. Horse tendon pounded with quartz and 1 per cent. saline swells to a jelly which, when spread on glass plates, dries to a substance resembling coarse unglazed paper. Pieces of this collagen " paper " were readily disintegrated by *Cl. welchii* type A filtrates and sera could be tested against a standard for their anti-disintegrating power, using pieces of collagen paper as indicator. Though testing by this method was sometimes easy and the results showed that the substances softening muscle and disintegrating collagen were the same (*i.e.* a collagenase), difficulties in preparing uniform collagen paper prevented the development of a satisfactory test until we hit on the idea of using the commercially available " hide powder ", which is a rich source of collagen. This hide powder was coupled with an azo- dye to give a bright reddish purple indicator (" azocoll "). When this was

incubated with *Cl. welchii* type A filtrates the disintegration of the collagen set the dye free into the liquid. Sera could then be tested for their power to prevent the diffusion of the dye from the azocoll during incubation with *Cl. welchii* type A filtrates.

Preparation of azocoll. Sieve 1 lb. hide powder (Baird and Tatlock) to 60 mesh; about 120 g. sievings should be obtained. To a solution of 0·575 g. benzidine in 100 ml. water containing 3 ml. conc. HCl cooled in an ice bath add slowly a solution of 0·45 g. sodium nitrite in 10 ml. water. Allow to stand for 10 minutes, then pour the tetrazotised benzidine into a chilled solution of 6·25 g. sodium acetate (CH₃COONa.3H₂O) in 500 ml. water. To the mixture add slowly with constant stirring a solution of 1·1 g. R-salt (sodium salt of 2-naphthol-3 : 6-disulphonic acid) in 100 ml. water, followed by 20 ml. N K₂CO₃. A brick-red dye is formed. In the meantime wash 80 g. of 60 mesh hide powder by repeated suspension in and filtration from 10 litres of water and finally re-suspend in 500 ml. water containing 30 ml. 2N K₂CO₃. Add the dye to the suspension in 6 equal lots at intervals of 10 minutes. The colour changes from brick-red to purple-red. After all the dye has been added, add 25 ml. 2N K₂CO₃ and allow the mixture to stand for ten minutes. Centrifuge or filter off the dyed hide powder and wash by re-suspending five times in 5-litre quantities of water. The wash liquors are coloured pink. Then re-suspend in about 300 ml. water, stir constantly and slowly add 1·7 litres of acetone. Repeat washing with acetone until acetone washings are no longer pink, filter off azocoll and remove residual acetone at 37° C. Sieve the azocoll to 60 mesh, dry over P₂O₅ under reduced pressure and keep in 5-10 g. amounts in rubber-capped bottles under nitrogen. Azocoll required for indicator should be suspended as needed in 1 per cent. Manucol IV * (300 mg. to 100 ml.). The Manucol is used to obtain an even suspension of the azocoll; in addition it may help to prevent the dye diffusing too far into the liquid when azocoll disintegrates.

Titration of sera. Make mixtures in Lambeth tubes of the test dose of filtrate at the level chosen (usually 2 units), amounts of serum differing by 10 per cent., and saline to make up to constant volume. Allow them to stand for half an hour, then add 1 ml. azocoll suspension to each. Incubate overnight in a water-bath at 37° C. (to prevent convection currents the water in the bath should reach the highest liquid level in the tubes). The end-point is taken as the tube which contains the maximum amount of antitoxin showing the development of a well marked red colouring above the azocoll. It has proved easy to use this test as a routine.†

Table III shows a comparison of the values obtained for sera using all three indicators. For nearly all sera the values obtained using muscle, collagen paper or azocoll as indicator are identical within the limits of the tests, suggesting that the same substance is responsible for the effects produced in all three.

Discordant sera

Of the 38 sera examined two—R 5603 and R 6514—show significant discrepancies between the values obtained in muscle and azocoll tests.

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* Manucol IV is a polymer of *d*-mannuronic acid (Allbright & Wilson, Birmingham). Occasionally a filtrate formed a gel with the Manucol; in such cases isotonic saline was used for suspending the azocoll.

† If sera are tested for very low levels of anti-κ activity, the presence of large amounts of serum in the test mixture may lead (in the presence of free κ toxin) to bleaching of the azocoll. Mixtures showing bleaching are always underneutralised.

There are at least three possible explanations: (1) that the muscle-softening and collagen-disintegrating substances are different and that the agreement in serum values using muscle and azocoll as indicators

TABLE III

Comparison of serum values against filtrate AG 835, using three different indicators

Serum	Serum values using as indicator		
	muscle	collagen " paper "	azocoll
R 8531	180	180	180
R 5434	50	58	55
A 16836	110	100	105
A 24422	180	170	170
R 7843	330	350	300
H 3296	500	600	400
H 3044	500	680	600
LX 251	800	700	650
R 5603	900	900	360
R 6514	150	...	90
Test dose . .	0·9 ml.≡2 ʌ units	0·9 ml.≡ κ units	0·5 ml.≡2 κ units
Minimum effective doses per test dose at 2 units	30	15	250
Sensitivity of serum value test *	±10 per cent.	±10 per cent.	± 5 per cent.
Accuracy of serum value test	±20 per cent.	±20 per cent. (irregular)	±10 per cent.

* Accuracy to which the end-point of a single titration can be read.

is fortuitous; (2) that the muscle-softening substance disintegrates collagen, but that another collagenase is present having no muscle-softening properties; (3) that R 5603 and R 6514 are non-avid and that the finely divided azocoll competes with them for toxin more effectively than pieces of muscle.

We therefore attempted to neutralise the muscle-softening substance in filtrate AG 952 with R 5603 and R 6514 and determined the values of sera against the partly neutralised filtrate, using azocoll as indicator. Table IV shows that there is no evidence for the existence of a second collagenase, for if there were, it would have been expected that the values of some of the sera against it would have been different from their anti-muscle-softening values. Nor has it been possible to demonstrate non-avidity in R 5603 or R 6514, for tests at higher levels show no tendency for the muscle and azocoll values to approximate.

The discrepancies in the values of R 5603 and R 6514 are therefore unexplained, for we find it difficult to believe that so many sera would

show concordant values in muscle and azocoll tests if the substances effective in these tests were different.

TABLE IV

Serum values (using azocoll as indicator) against unneutralised and partially-neutralised AG 952

Serum	Serum value against		
	filtrate AG 952	AG 952 partially neutralised with R 5603	AG 952 partially neutralised with R 6514
R 8531	180	180	180
R 5434	50	55	45
R 6712	120	100	120
R 6743	320	310	330
R 7843	300	300	250
GGC 3296	570	500	580
H 2917	800	700	700
Test dose . .	0·33 ml.≡2 κ units	0·8 ml.≡1 κ unit	0·8 ml.≡1 κ unit

Can α toxin disintegrate muscle?

It is easy to show that *Cl. welchii* type A filtrates in which all the α toxin has been neutralised still disintegrate muscle, while filtrates in which all the κ toxin has been neutralised have no muscle-disintegrating power, though large amounts of α toxin may still be present.

Histological examination of pieces of muscle or liver incubated with *Cl. welchii* type A filtrates shows that, if κ toxin is present, the reticulum surrounding the muscle fibres or liver trabeculæ is destroyed whether α toxin is present or not. If all the κ toxin is neutralised, no effect is produced on reticulum, however much α toxin is present.

Do types B, C and D of Cl. welchii produce κ toxin?

A few strains of types B, C and D have been examined. Of these only type C produced measurable amounts of κ toxin (0·83 ml. of filtrate≡$\frac{1}{2}$ κ unit by azocoll testing), while filtrates of type B and D cultures contained only traces.

Summary

1. *Cl. welchii* type A filtrates contain at least one additional toxin immunologically distinct from α toxin, θ toxin and hyaluronidase. This substance (κ toxin) is a collagenase which breaks down muscle by attacking its collagen and reticulin scaffolding and may therefore be responsible for the pulping of muscle seen in human gas gangrene.

2. The anti-κ values of sera can be determined using muscle, collagen " paper " or azocoll as indicator. A method for preparing azocoll is given.

3. A few sera show unexplained discrepancies between their anti-κ values determined against muscle and azocoll as indicators.

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LABORATORY FINDINGS IN CLINICAL DYSENTERY IN MIDDLE EAST FORCE BETWEEN AUGUST 1940 AND JUNE 1943

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MIDDLE EAST FORCE was, during the period covered by this communication, an extensive command, and had hospitals with their attached laboratories in Egypt, Sudan, Eritrea, Palestine, Syria, Cyprus, Libya, Cyrenaica, Tripolitania and Malta. The figures given in this paper are compiled from returns received from laboratories in all these countries, and can therefore be claimed to be a reliable cross-section of the dysenteric flora of this area. They were rendered in monthly reports submitted to the Deputy Director of Pathology, Headquarters, Middle East Force, between August 1940 and June 1943, when it was decided that it was no longer necessary to attempt the isolation of dysentery bacilli as a routine procedure in all suspected cases.

METHODS

The methods used in the isolation and identification of dysentery bacilli were those taught at the Royal Army Medical College, which have been described elsewhere (Boyd, 1939 40). For the isolation of the organism, McConkey's medium or litmus-lactose-bile salt agar medium was used. Sodium desoxycholate was not available until the end of this period and then only in limited quantities. Its use would probably have increased the percentage of isolations (though a controlled experiment showed that in the acute stages of bacillary dysentery its advantages are not so obvious as in the old or chronic case) but would have made no important difference in the percentages of the different types found. For serological confirmation of the identity of the dysentery-like organisms isolated, the following antisera (all supplied by the Emergency Vaccine Laboratory) were used —(1) Shiga, (2) Schmitz, (3) Sonne, (4) Flexner polyvalent 1 and 2.

Flexner polyvalent 1 serum was a mixture of sera prepared from Flexner types I (V), II (W) and III (Z) in quantities sufficient to give an approximate titre of 1 : 250 for each of these types. Flexner polyvalent serum 2 was a mixture of Flexner IV (103), V (P 119), VI (88-Newcastle Manchester) and Boyd I (170). The last of these was included because this organism had been found to be of relatively common occurrence in India. Other types identified in India (Boyd, 1932 and 1939 40) were deemed to be too rare to merit inclusion in routine diagnostic sera, but in order to maintain a check on their occurrence, instructions were issued for unidentified dysentery-like organisms to be sent to the Central Pathology Laboratory, where mannitol fermenters were investigated by the author and non mannitol fermenters by Major J. D. MacLennan.

Results

Table I is an analysis of the findings during the period under review. The figures include all cases investigated by laboratories, but do not represent the total incidence of dysentery in the force, as many cases were of necessity diagnosed on clinical grounds only.

Table I

Cases of clinical dysentery investigated between August 1940 *and June* 1943, *showing isolations and percentages*

	August to December 1940	1941	1942	January to June 1943	Total	Isolations as a percentage of total cases investigated	Percentages of various dysentery bacilli isolated
Total number of cases of clinical dysentery investigated	2381	22,578	31,991	8023	64,972		
B. dysenteriæ Shiga	97	1375	2696	348	4516	6·95	18·86
Schmitz	75	455	917	154	1601	2·46	6·68
Sonne	35	567	822	342	1766	2·71	7·37
Flexner I to VI and Boyd I	755	4533	7855	1609	14,752	22·71	61·59
Other non-mannitol fermenters	2	311	143	50	516	0·79	2·16
Other mannitol fermenters	92	354	330	24	800	1·23	3·34
E. histolytica	130	1070	1554	1709	3463	5·33	...

The total number of dysentery bacilli isolated was 23,951. The percentage of isolations (including *E. histolytica* 42·19 per cent.) is lower than that usually obtained in peace-time conditions, but this is the inevitable result of the "rush" which occurs during the dysentery season, when it is often impossible to examine more than one specimen from each patient or to devote more than half a plate of medium to each specimen. In practice, the isolation and identification of the organism is not in the majority of cases a matter of much importance to the physician. The experienced pathologist has no difficulty in making an accurate diagnosis of the type of dysentery (whether bacillary or amœbic) from the microscopic characters of the exudate, and no further differentiation is normally required for purposes of treatment.

It is of interest to compare these figures with those of the Army in India (table II). The Indian figures are from a series of 7339 strains identified in the years 1932–1935. They do not include "other non-mannitol fermenters", but as these were of rare occurrence, the percentages are not significantly raised. The close relationship between the two sets of figures is remarkable.

The vast majority of the "missed" cases were bacillary and not amœbic dysentery. The latter is rarely a self-limiting disease, and, although temporary remissions may occur in the absence of diagnosis

and specific treatment, recurring relapses sooner or later draw attention to the real nature of the disease. For this reason, the true incidence of amœbic dysentery is unlikely to be much greater than

TABLE II

A comparison between the percentages of dysentery bacilli identified in India, 1932-1935, and Middle East Force, August 1940-June 1943

	Shiga	Schmitz	Sonne	Flexner I-VI and Boyd I	Other mannitol fermenters	Other non-mannitol fermenters
Army in India, 1932-1935 .	14·3	5·5	10·9	62·3	6·9	No record
Middle East Force, August 1940-June 1945	18·86	6·68	7·37	61·59	3·34	2·16

that shown in table I, *i.e.* just over 5 per cent. of all cases. Its incidence was slightly higher in the Sudan and Eritrea than in Egypt and Palestine and tended to be greater in troops who had been in the country for some time.

Of the dysentery bacilli isolated, 94·5 per cent. were of recognised types and were readily identified by the standard antisera supplied by the Emergency Vaccine Laboratory. No attempt was made to identify the individual types of Flexner bacilli, nor are accurate figures available to show the proportions which fell into the groups covered by the Flexner polyvalent 1 and Flexner polyvalent 2 antiserum respectively. It can be said, however, that a very considerable number were in the latter group, indicating that these more recently described types are of common occurrence in the Middle East as in India.

Atypical strains

An account of the atypical non-mannitol fermenters has already been published by MacLennan (1945). Of the 800 mannitol fermenters which were not identified by the standard antisera, a total of 109 strains were received at the Central Pathology Laboratory for investigation.

Fifty-five of these were of types already described (Boyd, 1932) and occurred in the following numbers :—Boyd II (P 288), 13 ; Boyd III (D 1), 2 ; P 274, 28 ; P 143, 5 ; D 19, 7. Type P 274 has now been shown to have a wide distribution, always in association with clinical bacillary dysentery. Originally described from India, it has been found in the Middle East as noted above, in Australia (Rothstadt *et al.*, 1943), in America (Kuhns, 1943 ; Wheeler, 1944 ; and others), and recently a strain was isolated in the Scottish Command Laboratory. It is therefore proposed that this type should be known as Boyd IV. For convenience, but with less justification, P 143 may be called Boyd V, and D 19 Boyd VI. No evidence other than that they

have been isolated only from cases of dysentery can be produced regarding the pathogenic action of the last two types.

Eighteen others were found to be of one antigenic pattern, but subsequent investigation showed that they were not true dysentery bacilli, as they possessed a degree of motility. Their capacity to produce indol in peptone water excluded them from the salmonella group.

Antisera were prepared from several of the 28 residual strains, but only two of these reacted with strains other than the homologous organisms. The two types thus identified accounted for 10 strains, leaving 18 unidentified. By an unfortunate mischance one of these types (Rhemes), of which there were 4 strains, was lost.

The remaining type (1296/7), of which 6 strains were found, was passed on to Lt.-Col. A. E. Francis for further investigation. It was found to contain Flexner group antigen and therefore qualifies to take its place as a member of the Flexner group. Its detailed characters have been described in a separate communication by Francis (1946) on two new Flexner types, in which 1296/7 is designated *Shigella Flexneri,* type VIII.

An analysis of these results is given in table III.

TABLE III

Analysis of 109 strains submitted as atypical mannitol-fermenting dysentery bacilli

Type	No.
Boyd II (P 288)	13
Boyd III (D 1)	2
Boyd IV (P 274)	28
Boyd V (P 143)	5
Boyd VI (D 19)	7
Flexner VIII (strains 1296/7) . .	6
Strain " Rhemes " (lost) . . .	4
B. dispar types	8
Not true dysentery bacilli (motile) .	18
Unidentified	18

It is perhaps worthy of note that *B. alkalescens,* which by virtue of its biochemical reactions should have found its place in this series, is conspicuous by its absence.

SUMMARY

1. 23,950 strains of dysentery-like organisms were isolated and investigated in the Military Laboratories in Middle East Force between August 1940 and June 1943.

2. 94·5 per cent. were serologically identified by means of the standard sera supplied by the Emergency Vaccine Laboratory.

3. Of the " atypical " non-mannitol fermenters, 50 of a series of 109 were of types which have already been described.

4. A new Flexner type (of which only 6 strains were isolated) has been identified and will be described as *B. dysenteriæ* Flexner VIII.

My thanks are due to the many pathologists in the laboratories of the Middle East Force, without whose willing collaboration it would have been impossible to compile these figures, and to Sgt. J. Pilling, R.A.M.C., who assisted in the investigation of the " other " non mannitol fermenting organisms.

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TWO CASES OF MULTIPLE MYELOMA (PLASMO-CYTOMA) WITH SECONDARY DEPOSITS IN THE DURA MATER

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(Plates XXXI-XXXIV)

TUMOURS composed exclusively of typical plasma cells constitute one of the well recognised histological types of multiple myeloma ; indeed, according to most writers, they form the majority of medullary tumours. Those yielding visceral and soft tissue metastases and the extramedullary primary plasmocytomata are of greater rarity. The particular interest of the present cases, which are otherwise typical, lies in the occurrence of tumour deposits of considerable size in the cerebral dura mater. We have found but one other case of the kind reported in the literature (Carlisle, 1938) and the condition seems exceptional enough to warrant recording.

Case 1

Clinical history The patient, a single woman of 47, was admitted to the Leeds General Infirmary complaining of " rheumatism " and " pains all over ". The attacks of pain covered a period of one year, but for about two months prior to admission she had also complained of marked lassitude and loss of weight. In addition, there was a history of cough and evening temperature for 11 days before admission to hospital The attacks of pain were typical of the condition of myelomatosis and were marked by the same sequence of events in whatever site they occurred The pain, of sudden onset, was sharp and stabbing in character and continuous in one particular site for a matter of weeks , it would then disappear suddenly and be followed by freedom from pain for a similar period This in turn would be followed by an attack in some other site. The right and left chest, scapular and lumbar regions and both legs in the distribution of the sciatic nerve were the principal sites of attack Pain in the right chest was aggravated by the cough which developed latterly.

The patient became seriously ill a few days after admission, with a painful cough and evening pyrexia, and persistent severe pain in the lumbar region. Death occurred two weeks later, following a period of marked dehydration and cachexia.

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PLATE XXXI

Fig. 1.—Outer aspect of the cerebral dura mater (case 1), showing a semipedunculated circular tumour situated approximately over the middle of the longitudinal sinus and resembling an arachnoidal endothelioma in appearance and position. Another small nodule of tumour tissue can be seen above and to the right. (Slightly reduced.)

Fig. 2.—Inner aspect of the skull from case 1, showing the circular cavity, eroded in the bone, in which lay the tumour shown in fig. 1. Only the thinned outer table of the skull remains. Another small eroded area below and to the right corresponds to the smaller tumour mass in fig. 1. The specimen has been partly transilluminated. (Slightly reduced.)

PLASMOCYTOMA WITH DURAL DEPOSITS
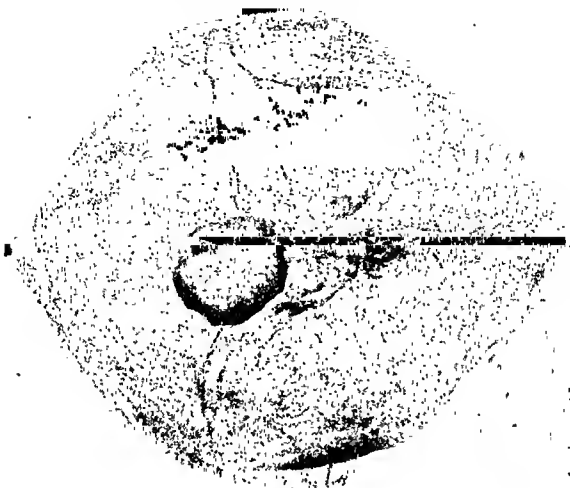
FIG. 1.
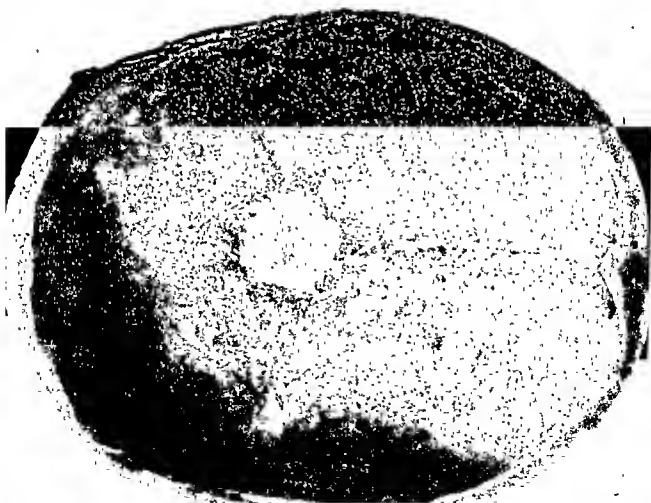
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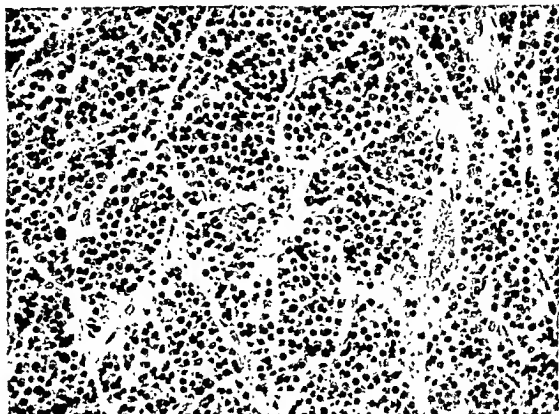
FIG. 3.—Section of dural tumour from case 1, showing the general arrangement of closely packed plasma cells in trabeculæ, separated by fine connective tissue septa containing capillary blood vessels.   × 250.
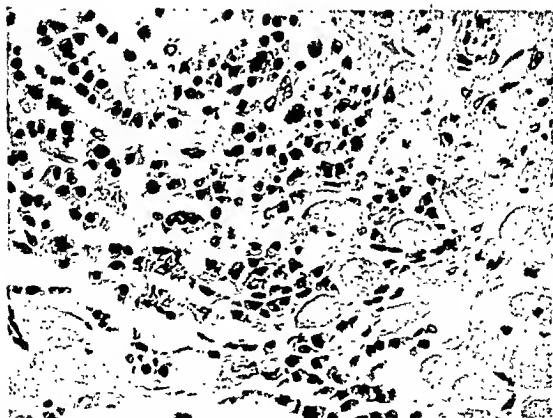
FIG. 4.—High power view of one margin of a costal tumour from case 1, showing diffuse infiltration of striped (intercostal) muscle by tumour cells.   × 400.

middle lobe and central portion of the lower lobe of the right lung and extensive bronchopneumonia of the left lung and base of the right. There was a long-standing calculous hydronephrosis on the left side, with compensatory hypertrophy of the right kidney.

Histology

The microscopical structure of the dural tumour is essentially the same as that of the osseous and glandular deposits. In general the tumours are characterised by great and uniform cellularity and an inconspicuous amount of stroma. The cells are closely packed and a trabecular arrangement is present in some areas of both medullary and dural tumours, the trabeculæ being separated from each other by fine connective tissue strands. In other areas no stroma is recognisable with routine staining and the cells are so closely packed as to show marked signs of mutual pressure. The tumour tissue is on the whole very vascular, with numerous small well formed blood vessels.

The type cell is the plasma cell, with characteristic structure and staining properties conforming to the four morphological criteria laid down by Marschalkó (quoted by Michels, 1931). In outline these cells are oval, round, or, when modified by mutual pressure, polygonal or rectangular ; they have opaque homogeneous cytoplasm and a clear crescentic perinuclear zone. The nucleus is relatively small and eccentrically placed, with blocks of chromatin arranged close to the nuclear membrane, giving the so-called " clock face " appearance. In hæmatoxylin and eosin-stained sections the tumour cells show faintly basophilic cytoplasm and some variation in depth of nuclear staining ; with the Unna-Pappenheim stain the cytoplasm yields the typical bright carmine colour of plasma cells. The vast majority of the cells are well differentiated. Mitotic figures are absent in most of the sections ; occasional binucleate forms are present, in some of which the usual chromatin arrangement is retained, while others show degenerative changes. A few cells with typical cytoplasmic outline and eccentrically placed nucleus give a bright red staining reaction with hæmatoxylin and eosin and display a dark-staining somewhat shrunken nucleus, but no degenerate forms with red-stained globules—Russell bodies—are seen. Vesicular cytoplasm characterises a small number of the cells.

In the dural tumour the most striking feature is the trabecular arrangement of the tumour cells (fig. 3). A few cells show degenerative changes, some presenting karyorrhexis and deeply acidophil cytoplasm, others large vacuolated nuclei and loss of cytoplasmic outline. The sacro-iliac tumour is particularly vascular and in it, large sheets of closely packed cells form a mosaic. In one of the costal tumours a nodule of neoplastic tissue has breached the fibrous periosteum and penetrated the adjacent striped muscle (fig. 4), while

tumour tissue is also seen lying free in the lumen of a small vessel (fig. 5).

Of the glands examined microscopically, the most advanced changes are seen in those of the cardiac group. Here the normal structure is entirely lost, the whole gland having undergone plasma cell replacement (fig. 6); only an occasional lymphocyte is seen. A gland from the lesser omentum shows marked sinus catarrh; the whole structure is very vascular and numerous mitoses are present. Some areas of a paratracheal gland on the other hand are packed with lymphocytes, with only a few plasma cells interspersed; other fields show the normal glandular structure in process of plasma cell substitution.

A specimen of sternal marrow obtained *post mortem* shows nothing abnormal.

Case 2

Clinical history. The patient, who died in 1932 at the age of 77, had been admitted to a mental hospital in 1884, when 28 years old, suffering from mania. In 1910 he was classified as suffering from dementia, but his physical state was good. In 1929 he had an infection of the right hand, with subsequent disorder of the metacarpo-phalangeal joint of one finger. In 1930 he complained of stiffness of the neck for which no cause was found. Examination of the chest revealed no abnormality and no abnormal constituents were found in the urine. He had a well marked intention tremor and his pupils reacted sluggishly. Weight 11 st. In January 1932 he had two slight attacks of hæmoptysis, after which he began to lose weight and to become sallow. The only abnormality found on examination was enlargement of the prostate, detected per rectum. By 21st May 1932 his weight had fallen to 7 st. 10 lb. By September he had become very weak and was running an occasional temperature, with tachycardia and slight dyspnœa. He died on 11th September.

Post-mortem examination

The body was extremely emaciated. On opening the thorax, a tumour the size of a hen's egg was found projecting into the interior of the chest (fig. 7). It sprang from the inner aspect of the left 8th rib in the mid-axillary line and was associated with a pathological fracture. A similar but smaller tumour was present in the right 5th rib. There were no tumours in the sternum. The lungs were the seat of a confluent bronchopneumonia. There were no deposits of growth in the lungs and no evidence of old tubercle. The aorta and coronary arteries were atheromatous but there was no evidence of syphilitic aortitis. The heart showed brown atrophy. The kidneys were small and the seat of much ischæmic scarring, with many old infarcts, while the capsules were adherent. The right adrenal contained a cortical adenoma 2 cm. in diameter. The digestive tract from the pharynx to the rectum presented no abnormality beyond patchy engorgement of the duodenum and small intestine. The testes were normal except for large bilateral hydroceles.

PLASMOCYTOMA WITH DURAL DEPOSITS

Fig 5 —Section from same tumour as fig 6   On the right a nodule of tumour tissue is seen within the lumen of a thin walled blood vessel   ×250
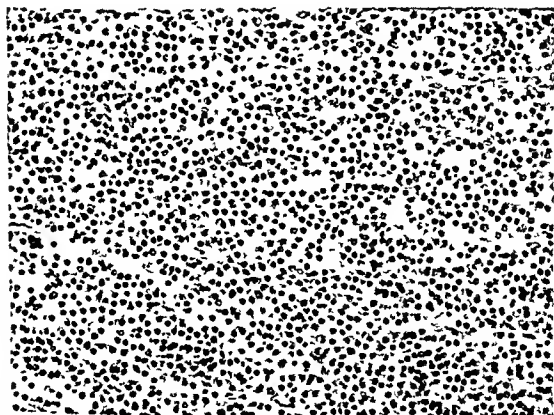
Fig 6 —Section of a gland of the cardiac group from case 1, showing complete plasma cell replacement of the normal lymphoid tissue   ×250

PLASMOCYTOMA WITH DURAL DEPOSITS

Fig. 7.—Above, the outer aspect of the cerebral dura mater (case 2), with two large tumours, one situated over the longitudinal sinus in an exactly comparable position to that in case 1. Below, the 8th rib, with a tumour arising in the mid-axillary line. Slightly reduced.

Fig. 8.—Outer aspect of the skull from case 2, showing the two large holes with irregular margins eroded in the bone by the dural tumours shown in fig. 7. Two smaller holes produced by smaller deposits are also shown. Slightly reduced.

Fig. 7
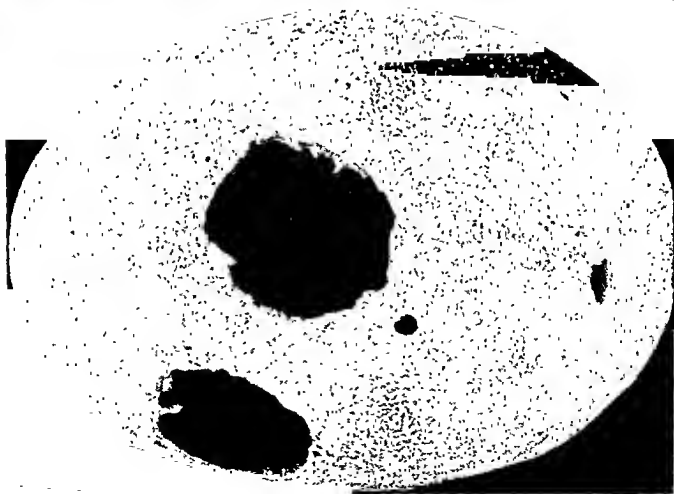
Fig. 8

figures, were the predominating feature, in the present cases the tumour cells are almost exclusively well differentiated plasma cells and mitoses are quite exceptional. Nevertheless, in spite of these distributional and histological differences, the tumours clearly possess comparable metastasising potentialities, including dural lesions with similar absorptive effects on the overlying calvarium. These facts support the view that, in myelomatosis, histological appearances with regard to the degree of differentiation of cells and number of mitoses bear little if any relationship to the capacity of the tumours for growth and metastasis. Hellwig (1943), considering prognosis in cases of plasma cell tumours, observes that microscopic appearances do not play a dominating role in predicting the clinical course, while Masson and Wolf classify plasma cell tumours into plasmocytoma and plasmosarcoma according to the occurrence or non-occurrence of metastases and not according to the histological features.

In the present instances the metastatic nature of at least the dural tumours would appear to be unquestionable. Although plasma cells are normally present in many internal organs, the occurrence of dural plasma cell tumours without visceral deposits is opposed to the idea that all plasmocytomatous lesions are part of a systemic change.

Blood-borne metastatic deposits of other tumours in the dura mater are not rare. They were present in 12 of Willis's (1934) personally observed series of 323 cases of malignant disease, and in a review of the literature this author found records of 51 cases of discrete dural metastases, the principal primary growths being mammary carcinoma (13 cases), pulmonary carcinoma (11 cases) and melanotic tumours (8 cases). The conception of blood-borne deposits in the present instances therefore seems reasonable.

The lymph-gland changes in case 1 present a more difficult problem. Primary plasmocytoma of lymph-glands is rare (Hellwig); we have encountered one striking example. The possibility of the changes being in the nature of a systemic response of fixed reticulum cells to the stimulus causing the primary tumours, as described by Parsons (1943) in experimental mice, must be considered. In case 1 sections of glands in the early stages of plasma cell replacement do not show any convincing intermediate forms between reticulum and plasma cells or between lymphocytes and plasma cells, nor any particular spatial relationship between proliferating reticulum cells and the collections of mature plasma cells present. The lack of immature cell forms and the occurrence of other metastases appear to support the view of a metastatic origin of the lymph gland tumours also. As the present advanced cases seem to add little to the already available evidence, it is not proposed to discuss whether the medullary tumours are to be regarded as systemic or metastatic in nature.

The erosive effect of the dural tumours on the calvarium is remarkable and in striking contrast to the hyperostosis which is so frequently seen where arachnoidal endotheliomas invade the skull.

Rather does the appearance resemble the bony absorption which occurs over normal or hypertrophied pacchionian bodies projecting from the outer aspect of the dura mater, with this difference that in the present instances the apertures in the skull look as if they had been drilled out or cut out with a knife (fig. 8).

The absence of Bence-Jones proteinuria is of little significance, since it occurs only in some 65 per cent. of cases (Donhauser and de Rouville, 1941). Renal tubular lesions of an obstructive nature are described by Bell (1933) and others as occurring frequently in cases of multiple myeloma, usually in the presence of Bence-Jones proteinuria. In the present case 2 the kidneys were unfortunately not examined microscopically, but the gross renal lesion present in case 1 was obviously of long standing and would appear to be unconnected with the neoplastic process.

Summary

Two cases of multiple plasmocytoma are described, one a woman of 47 in whom there were numerous medullary and lymph-glandular lesions and one large and one small deposit in the cerebral dural mater, the other a male lunatic aged 77 in whom there were two massive and two smaller deposits in the cerebral dura and deposits in two ribs. In this case the dural tumours had penetrated completely through the whole thickness of the vault of the skull, while in case 1 only the much thinned outer table remained over the larger growth. It is concluded that the dural tumours were due to blood-borne metastasis and were not part of a multifocal primary systemic change.

Only one similar case has been found in the literature.

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THE EFFECT OF TEMPERATURE ON NON-SPECIFIC INFECTIONS OF FISH

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IN the course of investigations of bacterial infections of fish and lower vertebrates, several workers have encountered organisms belonging to groups which are usually considered to be essentially saprophytic in habit

Epidemics of disease among roach and other fresh water fish were studied by Wyss (1898) and Babes and Riegler (1903), and in both instances the infecting organism was identified as Proteus An infection of frogs and fish was reported by Sanarelli (1891), and later Russell (1898) isolated a similar bacterium from a disease of frogs It appeared to be a member of the Pseudomonas group Hume Patterson (1903), from his studies of 'fungus disease" of salmon, concluded that the fungus represented a secondary infection and that the primary infecting organism was a "liquefying bacillus ' which he did not identify Cultures obtained from "fungus disease" of salmon, and exactly resembling those described by Hume Patterson, were examined by the author and found to consist of a mixture of a typical Proteus and *Pseudomonas fluorescens* The pigmentation of the latter organism was suppressed in the mixed cultures, except for an occasional clouding of the medium as reported by Hume Patterson Anderson (1909) examined lesions in fish taken from the vicinity of a sewer and isolated from them bacteria which he identified as Staphylococcus and typical *Escherichia coli* Anderson apparently considered that the organisms responsible for the disease were derived from the sewage Williamson (1929), in a review of bacterial infection of the lower vertebrates, concluded that in certain circumstances saprophytic water bacteria might become pathogenic for these animals Calmette (1922) expressed similar opinions with regard to saprophytic Mycobacteria

Preliminary observations

In the course of numerous routine examinations of fish in connection with the laboratory services under the Diseases of Fish Act, the writer has many times isolated what appeared to be saprophytic water bacteria from pathological conditions in salmon and fresh-water fish These appeared to belong mainly to the Proteus, Pseudomonas and Achromobacter groups, although representatives of other genera were also encountered

From a number of salmon, organisms with the characters of the

Proteus group have been isolated, in some cases apparently associated with lesions. Similar organisms have been found in the tissues of healthy trout taken from hatcheries in the course of examination for carriers of *B. salmonicida*. These frequently differed from the classical strains of Proteus in their ability to ferment mannitol.

Goldfish have been frequently observed to carry large numbers of bacteria, *Ps. fluorescens* being exceedingly common. For this reason these animals were first chosen for the experimental work recorded in this paper.

It had been noticed, in relation to other diseases of fish, that temperature is of prime importance in the occurrence and severity of the disease (see Second Interim Report, Furunculosis Committee, 1933), and accordingly it was decided to test the effect of varying temperatures upon the incidence of the non-specific infections referred, to above. Goldfish were considered specially suitable from this viewpoint also, as they are capable of existing comfortably at temperatures over a considerable range.

Experimental observations

Experiment 1

In the first experiment 47 goldfish were used. A few drops of peritoneal fluid were aspirated from each with a sterile hypodermic syringe, cultures were made on yeastrel agar and incubated at 22° C. A bacterial growth was obtained in this manner from 21 fish; the remaining 26 appeared to carry no cultivable bacteria in the peritoneal cavity. The organisms isolated were mainly proteolytic Gram-negative bacilli of the Proteus, Pseudomonas, Chromobacter and Achromobacter groups, diphtheroid bacilli and Micrococcus, both the latter being of low fermentative power and non-proteolytic; a proteolytic Sarcina and a Staphylococcus were also isolated from time to time. The growths were usually mixed, although occasional pure cultures were obtained.

Infected and uninfected fish were each divided into two groups, one group of each category being placed in a thermostatically controlled tank at 10° C., the other in a similar tank, the temperature of which was gradually raised over 2 or 3 hours to 23° C. and maintained at that temperature.

The fish were distributed as follows :—

Tank 1. 23° C. . . . . 11 infected fish
　,,　2. 23° C. . . . . 10 uninfected fish
　,,　3. 10° C. . . . . 13 infected fish
　,,　4. 10° C. . . . . 13 uninfected fish

Each tank was aerated by means of a small jet of water directed continuously upon the surface. The fish were fed on alternate days with dried Daphnia.

The tanks were examined at frequent intervals throughout the day and fish which died were at once removed and cultures made from the peritoneal fluid. In all cases a profuse growth was obtained.

At the end of twelve days all surviving fish were killed and cultures were made from the peritoneal fluid. These were incubated at 22° C. and the growths obtained were recorded as " profuse " or

" slight ", the former indicating numerous colonies or a confluent growth, the latter a small number of discrete colonies. Judged by

TABLE I

Time of death of fish (expt. 1)

| | Temp. | Day of experiment | | | | | | | | | | | | Total deaths |
		1	2	3	4	5	6	7	8	9	10	11	12	
Tank 1	23° C.		1	1			1		1					4
„ 2	„		1											1
„ 3	10° C.										1			1
„ 4	„											1		1

the same criteria the amount of growth in the original cultures from the living fish was usually slight in proportion to the comparatively large inoculum of peritoneal fluid used in those cases.

Cultures from surviving fish. The bacteria in these cultures were much the same as those obtained originally from the fish, but the proportion of Gram-negative bacilli was somewhat greater, particularly in those with a heavy infection and including those dying during the experiment, all of which were heavily infected, as already stated. These results indicate that, when infected fish are maintained at the higher temperature, the degree of infection increases and may prove fatal in a small proportion of cases. That the increase of temperature

TABLE II

Result of cultures from surviving fish (expt. 1)

	Profuse growth	Slight growth	No growth
Tank 1	3	3	1
„ 2	0	0	9
„ 3	1	8	1
„ 4	1	4	7

alone is not the cause of death is shown by the fact that the fish in tank 2, which was also at a temperature of 23° C., showed a death-rate identical with that among the fish in the two cold tanks (table I). The degree of final infection in the previously infected fish kept in the cold tank 3 remained moderate : the uninfected fish in the warm tank 2 remained uninfected (table II). In the cold tank 4 the fish showed some tendency to acquire new infection from the tank water, which always contained a large number of bacteria.

Experiment 2

The experiment was then repeated with a larger number of fish. Out of 103, 67 were found to be infected, 36 uninfected. These were

divided as before and placed in tanks at 10° and 23° C., numbered as in the previous experiment. The results are shown in table III.

TABLE III

Time of death of fish (expt. 2)

Day of expt.			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Total
Tank 1	23° C.	32 infected fish	1	2	2	1		1	1									1			9
,, 2	,,	15 uninfected fish						1													1
,, 3	10° C.	29 infected fish																	1		1
,, 4	,,	21 uninfected fish																			0

In all cases profuse growths were obtained in cultures made from dead fish.

By comparison with later experiments (*vide infra*), made without prior examination of the fish, it appears probable that the wound in the peritoneal wall made by the insertion of the hypodermic needle may have contributed slightly to the higher death-rate in both the preceding series.

On the 19th day the survivors were killed and cultures made from the peritoneal fluid (table IV).

TABLE IV

Results of cultures from surviving fish (expt. 2)

	Profuse growth	Slight growth	No growth
Tank 1	2	2	19
,, 2	1	1	12
,, 3	10	7	3
,, 4	1	3	16

Comparison of the figures for tanks 1 and 3 and with the corresponding figures for the previous experiment shows that the increased degree of infection of the previously infected fish (previously observed to occur in the course of approximately 12 days at the higher temperature) is followed in the course of the succeeding week by a decrease, the surviving fish apparently clearing themselves of infection. During the same time, the relationship between host and parasite in the cold tanks remains unaltered and the uninfected fish at the higher temperature acquire no new infection.

Experiment 3

In order to confirm these results still further, 146 fish were taken and divided arbitrarily into two groups without previous examination. As before, they were placed in tanks at 23° and 10° C. respectively. These fish were all in exceptionally good condition, and since there

was no trauma from an initial examination, as in the previous experiments, the actual death-rate was low. Two fish died in the warm tank, one on the 7th and one on the 11th day. None died in the cold tank.

Samples of equal numbers of fish from each tank were taken at intervals. They were killed and cultures were made from the peritoneal fluid. The results are shown in table V.

TABLE V

Growth in culture from sample fish (expt. 3)

Day of expt.	No. of fish from each tank	Growth on culture					
		Warm tank (23° C.)			Cold tank (10° C.)		
		Profuse	Slight	None	Profuse	Slight	None
10	10	4	2	4	3	2	5
20	12	3	2	7	2	5	5
30	24	4	3	17	6	9	9
45	15	0	5	10	3	7	5
55	12	0	1	11	1	2	9

These results may be expressed as the ratio of infected fish per unit of uninfected fish in each sample :—

Date of sample	Warm tank	Cold tank
Day 10	1·5	1·0
„ 20	0·7	1·5
„ 30	0·4	1·7
„ 45	0·5	2·0
„ 55	0·1	0·3

With the exception of the last figure in the cold tank series, this shows, in the warm tank, a gradual decrease in infection and in the cold tank a corresponding increase. As the bacterial content of the water was high throughout in both tanks, it would appear that initial infection or reinfection of healthy fish takes place more readily at the lower temperature.

Experiment 4

To test the effect of a temperature intermediate between 10° and 23° C., cultures were made from fifty living fish, and the infected ones, thirty-three in number, were placed in a tank at 15° C. Sample fish were killed at intervals in the course of the next month. There were no spontaneous deaths and no reduction of infection. It may therefore be assumed that the critical temperature for these phenomena lies between 15° and 23° C.

Experiment 5

The ability of fish to clear themselves of infection more rapidly at high than at low temperatures was confirmed by an experiment originally intended to test the pathogenicity for fish of hæmolytic streptococci isolated from an infected throat in the human subject. Twenty-four large goldfish were injected intraperitoneally with 0·5 c.c. of a dense emulsion of a 24 hours' culture of a group C hæmolytic streptococcus. Eight fish were placed in a tank at 10°, eight at 20° and eight at 30° C. Only one death occurred, in the tank at 20° C. immediately after injection. The other fish showed no signs of ill-health and it is improbable that the solitary death was due to the streptococci. These organisms were present in the tissues, but in no greater quantity than in the first healthy samples. At the end of a week two fish were killed from each tank and cultures made from heart blood, liver, kidney, spleen and peritoneal fluid. Similar samples were taken at intervals. The results are shown in table VI. At the

TABLE VI

Streptococci in the organs of experimentally infected goldfish

Temp. of tank	10° C.					20° C.					30° C.				
Organs	H	S	L	K	P	H	S	L	K	P	H	S	L	K	P
7 days	+ +	+ +	+ +	+ +	+ +	−	+ +	+ +	+ +	+ +	−	+	+	+	+
16 ,,	+ +	+ +	+ +	+ +	+ +	−	+	+	+	+	−	−	−	−	−
30 ,,	+	+	+	+	+	−	−	+	−	+	−	−	−	−	−

H = heart   K = kidney
S = spleen   P = peritoneal fluid
L = liver

end of thirty days streptococci were thus still present in large numbers in all the organs of the fish in the coldest tank; at 20° C. the fish cleared themselves slowly and at 30° C. more rapidly. At both 20° and 30° C. the blood-stream, if ever infected, was most rapidly cleared.

Discussion

It appears from the data recorded above that fish may become parasitised, sometimes heavily, by saprophytic water bacteria. Normally this causes little pathological effect, but under exceptional circumstances disease and even death may result. There appears to exist, at low temperatures, a balance between the aggressive action of the bacterium and the defensive powers of the host. Under such conditions the infected fish might be likened to a healthy carrier of a specific pathogenic organism. When the temperature is raised the balance is upset, probably by changes in the metabolic activity of both host and parasite. In these circumstances, if the fish is previously

uninfected its defences will be so greatly increased that it becomes completely resistant to infection. If, on the other hand, bacterial parasitism has already become established, the bacteria tend at first to increase and a proportion of the fish may die. In time, however, the surviving fish succeed, in many cases, in eliminating the bacteria from their bodies.

These points have been demonstrated in a striking manner by the investigations of Markoff and Jatschewa (1939) on disease of trout in a Hungarian lake, the main infecting organism being Proteus. These writers draw attention to the fact that the fish had previously been subjected to considerable changes of temperature without ill-effect, but that after the commencement of the infection among them the severity of the disease was greatly accentuated by increased temperature.

The survival of hæmolytic streptococci in the tissues of fish at low temperatures is paralleled by observations made by Harkins (1927), Brunner (1937-38) and Hettche (1937-38), who reported the survival in fresh-water fish of *Erysipelothrix rhusiopathiæ* derived from the offal of infected pigs. On occasion this has been reported as resulting in the infection of persons handling the fish.

Steinhaus (1940) quotes numerous accounts of the survival of bacteria, both saprophytes and human pathogens, in Arthropoda.

The adoption of a parasitic and even a pathogenic habit by an otherwise completely saprophytic species must be of basic importance in the study of the nature of bacterial disease in animals generally. It is notable that bacteria provide, similarly, the only examples of parasitic species which can readily be constrained to adopt a saprophytic existence in artificial culture.

Summary

1. Fresh-water fish may become parasitised by what are ordinarily saprophytic water bacteria.

2. These do not normally give rise to pathogenic effects, but may on occasion cause disease or death.

3. Experiments on goldfish have shown that a rise in temperature from 10° to 23° C. will upset the balance between the host and these ordinarily harmless parasites.

4. When this occurs there is at first an increase in the number of bacteria in the tissues of the fish, which may result in death. If the fish survives, the number declines and the tissues become cleared of infection.

5. Because of this increase in the anti-bacterial defences of the fish, they are enabled, at the higher temperature, to withstand initial infection more successfully.

6. At low temperatures the efficiency of these defences is so low that even organisms which appear devoid of pathogenic power towards

fish, *e.g.* hæmolytic streptococci, are not eliminated from their systems over a period of some weeks, whereas at higher temperatures their elimination is rapid and complete.

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SKIN AND NOSE CARRIAGE OF BACTERIOPHAGE TYPES OF *STAPH. AUREUS*

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SEVERAL workers have reported the association of the carriage of *Staph. aureus* on the skin of the back of the wrist with its carriage in the nose, and Gillespie *et al.* (1939) demonstrated the serological identity of the nose and skin strains; the numerical association was confirmed by work reported in 1944 (Miles *et al.*). It has now been possible to extend the study by the use of the phage-type filtrates developed by Wilson and Atkinson (1945).

METHODS

Bacteriological methods. As in the previous investigation, nasal samples taken with broth-moistened swabs through both anterior nares were plated on horse-blood agar and examined after two days' incubation at 37° C. In the study of 50 students recorded below, Fildes's agar plates were used for nose and skin samples. The skin was sampled with a moist swab, which was rubbed over an area about 3 cm. in diameter for 10 seconds. The swab was inoculated on half a blood agar plate and into broth, which was incubated for two days and then subcultured on blood agar, incubated anaerobically for one day and aerobically for a further 24 hours. The anaerobic incubation was designed to suppress the growth of aerobic spore-bearing organisms, which sometimes obscured the staphylococcal colonies. In the later stages of the investigation it was found that this end was better attained by anaerobic incubation of the broth culture; the plate subculture could then be incubated aerobically without fear that it would be overgrown. All colonies resembling staphylococci were tested for coagulase activity as in the previous work.

Many of the strains were phage-typed before the full battery of filtrates described by Wilson and Atkinson was available and it is therefore impossible to assign all of them to the " types " defined in that paper. Moreover, many of the strains, although lysed by some of the filtrates, did not fall into any of the defined types. These facts are of no importance where the identity or otherwise of two strains tested on one day is in question, but they do affect the construction of the frequency distribution of the types that is needed to assess the probability of chance duplication. The distribution given below (table I) is in terms of the various combinations of phage reactions, and later work may show that some of the combinations here considered distinct are in fact no more than variants of a single type. This would mean that the probability of chance duplication calculated below is too low, but it will be clear that a considerable number of types would have to be affected to modify the general conclusions.

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Subjects studied. Data from three groups of people are presented: (*a*) out-patients attending hospital for the first time with newly inflicted wounds which showed no clinical sign of infection; (*b*) 50 volunteer male medical students aged about 18-23, 19 in their clinical and 31 in their preclinical years of study; (*c*) 33 members of the hospital staff who were not, in their ordinary work, likely to handle infected wounds.

RESULTS

The association of nose and hand carriage

Strains of *Staph. aureus* isolated from the nose and skin of the hand of 65 out-patient double carriers were phage-typed. Both nose and skin strains were untypable in 18 cases; in 11 only one strain was typable and in 36 cases both strains were typable. In 31 of these 36 instances of typable pairs, the skin strain was identical with that from the nose; in five instances the types were certainly different. Clearly the nasal strain was also found on the skin in the majority of double carriers; the discrepancy of the types in some might be due to the presence on the skin or in the nose of an additional (adventitious) type. To investigate this possibility, 10 colonies from the primary plates of each of five nasal cultures were typed and in each case all the strains were either of the same type or untypable. However, two swabs taken from different areas of the skin of the hands of 22 subjects gave the following results: in 10 both strains were typable and the same; in 4 both were typable but different; in 3 both were untypable; and in 5 one strain was typable, the other not. It is clear, therefore, that, as Fisk and Mordvin (1944) showed in one instance, more than one strain may be present on the hand; had several colonies been typed as a routine, it is possible that a greater proportion of double carriers of identical types might have been discovered.

The duplication of phage types in the nose and on the skin in a high proportion of double carriers strongly suggests that carriage on these two sites is interdependent. Duplication of a given type might occur by chance, the probability of the occurrence depending on the frequency of the various types in the population at large. For instance, if one type constituted 50 per cent. of all the strains isolated, chance duplication would be common, whereas the chance duplication of a rare type would be unlikely. We might estimate the frequency of the various types by classifying all the strains that have been phage-typed. This, however, would be misleading, for if similarity of type in fact holds for the strains isolated from one person, then the frequency of any one type will be partly determined by the number of positive samples from each subject, and the resulting frequency distribution will not represent the frequency of the types in a random sample of staphylococci. A more reliable frequency distribution is shown in table I, consisting of the typable staphylococci isolated from

the noses of 127 subjects and from the skin of 34 others who had no
nasal strain, *i.e.* not more than one strain is included from any one
patient. We may assume that the strains isolated from the nose

TABLE I

Distribution of frequencies of phage reactions * in
105 typable strains of Staph. aureus

(1) Frequency f	(2) No. of distinct phage-reaction types with frequency f t	(3) $f \times t$	(4) $f^2 \times t$
1	24	24	24
2	13	26	52
3	3	9	27
4	4	16	64
6	3	15	75
7	1	7	49
8	1	8	64
		$N = 105$ $N^2 = 11025$	$(f^2 t) = 355$

* See p. 259 for discussion of the term " phage reaction "

or from the skin of these different subjects will be independent, since
the subjects were not members of a limited community through
which one type of staphylococcus might be expected to have spread.
One hundred and five strains were typable, 56 were not.

Miles (1946) has shown that, if it is assumed that the frequencies
of types in a sample of the sort given in table I are proportional
frequencies, the probability (p) that two strains drawn at random
from the population will be of the same type is given by :

$$p = t_1(1/N)^2 + t_2(2/N)^2 + t_3(3/N)^2 \ldots \text{ etc.}$$
$$= 1/N^2 \Sigma(f^2 t). \qquad\qquad\qquad (1)$$

where N is the number of strains in the sample, t_1 the number of
distinct types each having a frequency f of one, t_2 the number with a
frequency of two, etc., and $\Sigma(f^2 t)$ the summation of $(f^2 t)$ for all values
of t. In a sample of n double carriers, the number of duplications to
be expected on a chance basis is np, and the standard deviation of this
estimate is $\sqrt{npq}$, where $q = (1-p)$. When p is small compared
with q, the frequencies of chance duplications conform to a Poisson
distribution, and the standard deviation of np is then $\sqrt{np}$.

Substituting in equation (1) the frequencies of distinct types
recorded in table I, we have $p = 355/11025 = 0.03$; *i.e.* the
probability of chance duplication of types in our observed population
was 0.03, and since we observed 36 double carriers of typable *Staph.
aureus* we would expect duplication to occur by chance $36 \times 0.03 = 1.08$
times. Since p is small compared with $1-p$, the standard deviation

of $36p$ is $\sqrt{36p} = \pm 1.04$. The observed number of type duplications was 31, an excess of 29.92 over the number expected on a chance basis. This excess, being over 20 times the standard deviation of the expected number, is highly unlikely to be a chance effect. The validity of this deduction is probably obvious from simple inspection of the low frequencies of many distinct types in column (2) of table I. The calculations have, nevertheless, been displayed in full as an example for application to more nearly borderline cases, where the distribution of types in the population at large is such that there is a higher probability of chance duplications.

Clearly the typing results, like the serological typing results of Gillespie *et al.*, confirm the inference drawn from the study of numerical associations : that the skin staphylococci are probably derived from the nose. Miles *et al.* gave reasons for thinking it unlikely that the nose strains were derived from the skin.

Source of skin staphylococci in persons not nasal carriers

Although there is a high degree of association between the nose and skin carriage of *Staph. aureus* (see Miles *et al.*), there are a number of skin carriers whose noses fail to yield the cocci. It may be that in these cases the staphylococci do not represent self-contamination from the nose, but, alternatively, that they come from the nose, where carriage is intermittent and the coccus was not isolated on the day of sampling. If these subjects' noses were swabbed on other days and proved positive, it would be possible to demonstrate a closer association of nose and skin carriage. To test this point, the nose and wrist of 22 persons were swabbed weekly on eight consecutive weeks, giving 176 pairs of nose-skin samples. *Staph. aureus* was isolated from the skin on 82 occasions, and on only 18 of these was it found on the skin but not in the nose. These 18 double samples came from nine people, whose carrier history is shown in fig. 1. Clearly the first two subjects were persistent nasal carriers, and we failed to isolate the staphylococci from the skin on one day; the third subject was a somewhat less persistent carrier, but the fact that all the nose and skin strains were untypable does suggest that they may have belonged to one as yet undetermined type. Of the other six, three yielded staphylococci from the nose on at least one occasion : in two cases (5 and 6) both nose and skin strains were untypable, while case 4 yielded a variety of types and, except on one day, there was no evidence for nasal contamination of the skin. The last three were never shown to be nasal carriers and we must presume that their skin staphylococci came from somewhere other than their own noses. In sum, therefore, six of the nine skin carriers were intermittent nasal carriers, although in three contamination of the skin from the nose was not demonstrated unequivocally by identity of the phage types.

Persistence of Staph aureus *on the hand*

In studying the carriage of *Staph aureus* in the nose, we showed that our results were most readily interpreted on the supposition that persistent carriage was common. The data from the hospital staff swabbed over a period of two months now show that the same is
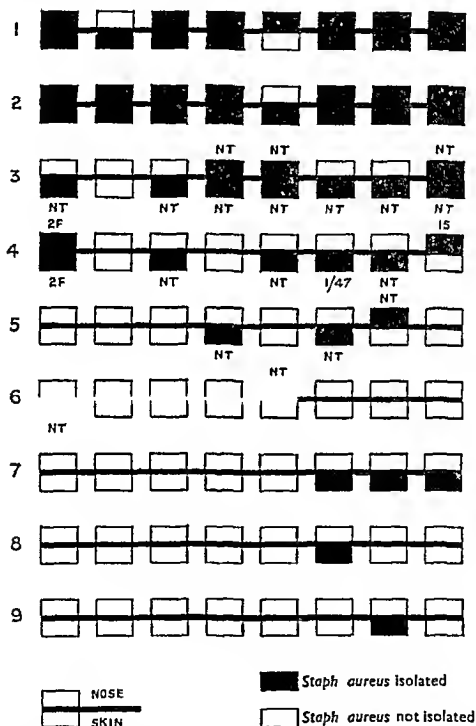
Fig 1.—Carrier histories of the nine subjects who on one or more occasions, yielded *Staph aureus* from the skin and not from the nose. Each block refers to one pair of weekly swabs. The small numbers refer to phage types, N T = untypable

true of wrist carriage. Applying the method described in the previous paper (Miles *et al*) to 33 persons swabbed on six consecutive weeks, we obtain the result shown in table II. The average carrier rate is 52 per cent. There is a marked excess in the observed distribution of subjects having 0 or 1 and 5 or 6 positive skin swabs over that

which would be expected if carriage was a matter of chance, and the probability of carriage in any one person was 52/100 (given by the binomial expansion of $(0\cdot52+0\cdot48)^6$). The value for χ^2, 71·8, gives $P = <0\cdot001$.

TABLE II

Persistence of skin carriage of Staph. aureus

No. of weeks positive	No. of subjects	
	observed	expected
6	3	0·65
5	10	3·61
4	5	8·34
3	0	10·26
2	4	7·11
1	7	2·62
0	4	0·41
Total	33	33·00

$\chi^2 = 71\cdot8$; $n = 5$; $P = <0\cdot001$.

A number of strains from persistent carriers have been typed. All the strains, isolated at weekly intervals from the skin of five of seven carriers swabbed 4-7 times, were identical; those from the sixth were all untypable, while the remaining carrier yielded at least three different strains from five samples. Similarly, of six persistent nasal carriers swabbed 3-8 times, four showed the same type on all occasions, a fifth showed three untypable strains and the sixth one discrepant strain out of eight typed.

Carriage of Staph. aureus on the skin of the body other than the hand

In assessing the importance of the nose as a source of staphylococci in wounds of the hand, we tested the back of the wrist for skin carriage as the part of the hand least likely to be contaminated by bacteria from objects handled, although, as previous work had suggested, readily contaminated from the nose. For a full appreciation of the staphylococcal risk for wounds on other parts of the body, it was clearly desirable to estimate also the carriage on areas other than the hand, and to find whether, in parts less obviously exposed to the risk of nasal contamination, the skin carriage of staphylococci was still related to nasal carriage. Swabs were therefore taken from the nose and from 11 different skin sites, each about 3 cm. in diameter, in 50 male medical students. No fewer than 35 of the 50 carried *Staph. aureus* on at least one site, though carriage on more than two sites was relatively rare and only five carried on more than four sites (fig. 2). The different sites and the frequency of carriage on each

of them are shown in table III: the back of the wrist was positive twice as often as any other site, a fact which may well reflect the
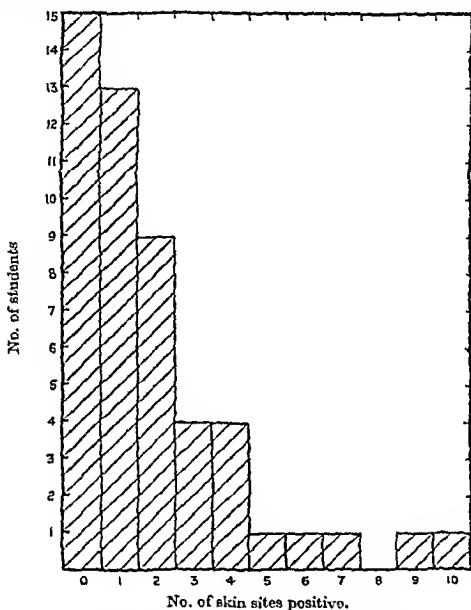
Fig. 2.—The extent of skin carriage of *Staph. aureus* on 50 students.

ease with which the wrist is contaminated, both from the subject's nose and externally from objects handled.

TABLE III

Frequency of carriage of Staph. aureus *on various skin sites in 50 young adults*

Site	Total no of carriers	No of profuse carriers
Back of wrist	20	14
Volar surface of forearm	10	9
Axilla	4	1
Middle third of sternum	6	4
Middle of abdominal wall	8	3
Middle of inguinal fold	9	4
Middle of front of thigh	8	5
Front of ankle	8	3
Back, over sacrum	7	3
Back of chest, middle	6	3
Back of neck	10	3

Nineteen (54 per cent.) of the 35 skin carriers were also nasal carriers, compared with three of the 15 non-carriers ($d = 34$ per cent.; $S.E._d \pm 15\cdot4$ per cent.; $t = 2\cdot2$; $P = 0\cdot02\text{-}0\cdot05$). In this study the nose swab was cultivated in the same way as the skin swabs and closer analysis reveals that 18 of the 22 nasal carriers yielded *Staph. aureus* from the direct plate and that all of these showed *Staph. aureus* on at least one skin site. Of the four nasal carriers yielding the coccus only from the broth subculture, only one was demonstrably a skin carrier. Furthermore, skin carriage was more extensive in nasal carriers than in non-carriers : the nasal carriers had an average of $2\cdot8$ skin sites positive, the non-carriers only $1\cdot2$ sites. Thus, as with wrist carriage, there is a clear indication that skin carriage over the whole body is associated with nasal carriage.

The skin and nose staphylococci from 10 students were typed; all except two of the 18 strains isolated from seven of them were identical in phage type with the nasal strain from the same man; one of four skin strains from one man was untypable and a single skin strain from another differed in type from the nasal strain. Of two other students, one yielded 10 strains from various parts of his body, all of which were lysed by filtrates 6 and 47, while his nasal strain was lysed by filtrates 6, 47 and 29 A ; the other, with five sites positive, yielded untypable strains from his nose and three skin sites and typable strains from two skin sites. One non-nasal carrier with eight skin sites positive gave identical staphylococci from seven, the eighth being untypable. It is clear that extensively contaminated skin usually carried one phage type of staphylococcus, usually identical with the strain isolated from the nose.

The development of bacteriophage typing gives much greater precision to this survey than was possible when the previous report was written. The evidence now presented fully supports the findings of Gillespie *et al.* as to the importance of self-contamination from the nose in the establishment of the skin carrier state. The great frequency with which nasal carriers contaminate their skin is also noteworthy. Thus among 50 students all but three of the 22 nasal carriers yielded *Staph. aureus* from some area of skin, and the three that did not yielded only a scanty growth from the nose ; of the 28 not shown to be nasal carriers, only 16 yielded *Staph. aureus* from the skin. Similarly, of 22 members of the hospital staff observed for eight weeks, 18 showed staphylococci in the nose on one or more occasions and only four of these never carried staphylococci on the wrist.

Frequency of skin carriage of Staph. aureus *in relation to sampling technique*

The carrier rates for the groups so far discussed were obtained by sampling about 7-10 sq. cm. of skin. From 28 patients we took a swab of this sort from the wrist and then swabbed the whole surface

of the hand with another swab. Six of the 28 wrist swabs and 19 of the whole hand swabs yielded *Staph. aureus* ; none of those positive by the first method was negative by the second. The same increase in the number of positives is shown by a comparison of the rate from wrist swabs of 256 subjects—25·4 per cent.—with that derived from 100 strictly similar subjects whose whole hand was swabbed, namely 56·0 per cent.

In the same way that the statement " the average nasal carrier rate for *Staph. aureus* is in the region of 50 per cent." does not give a complete picture of the magnitude of the nasal reservoirs of staphylococci, since 80-90 per cent. of people are found to be carriers at some time during a 10-week period, so the statement in the previous paper (Miles *et al.*) that the wrist carrier rate is 10-20 per cent. must be read in conjunction with the method adopted for sampling and for culture. By the use of broth enrichment the proportion of demonstrable wrist carriers was increased from 10·9 to 25·4 per cent. of 256 cases, and by increasing the area swabbed the proportion was again increased, as noted above, from 25 to 56 per cent. Similarly, if the area swabbed is increased by sampling a number of small areas distributed over the body, the same rise is apparent : the wrist carrier rate of our 50 students was 40 per cent., while the total carrier rate, determined from 11 skin sites, was 70 per cent. Lastly, if the wrist is sampled repeatedly over a number of weeks, the total carrier rate is increased : of 22 people swabbed on eight weeks, an average of 10·1 carried on any one week, but in the whole period of eight weeks, 18 of the 22 were carriers. We made a very similar observation to this in studying the persistence of nasal carriage : among 46 people swabbed on eight weeks, the average carrier rate on any one week was 63 per cent., while the total carrier rate determined over the eight weeks was 89 per cent. The same phenomenon has been observed by other workers studying the carriage, for example, of hæmolytic streptococci, meningococci and pneumococci (Straker *et al.*, 1939 ; Schwentker *et al.*, 1943 ; Kuttner and Krumwiede, 1944 ; Phair and Schoenbach, 1944).

SUMMARY AND CONCLUSIONS

Bacteriophage typing has been used to study the association of nose and skin carriage of *Staph. aureus*. The coccus was isolated from the hand and nose of 65 patients ; both strains were typable in 36 cases and were of the same type in 31, a proportion considerably in excess of that to be expected on a chance basis.

Data from 22 subjects whose nose and skin were swabbed on eight consecutive weeks suggest that some of those people who, when tested on one occasion only, yield *Staph. aureus* from the skin but not from the nose are intermittent nasal carriers, and that their skin is probably contaminated from the nose.

Wrist carriage of *Staph. aureus*, like nose carriage, may often be persistent over a number of weeks and the type carried is usually constant.

Swabs taken from 11 skin sites distributed over the body showed 70 per cent. of 50 students to be carriers of *Staph. aureus* on at least one site ; carriage on more than two sites was uncommon. Carriage on the skin as a whole was associated with nasal carriage, and in the majority of cases the skin strains were all of one type and were of the same type as the nasal strain.

A numerical value for the skin carrier rate must always be interpreted in relation to the methods of sampling and culture used : single wrist swabs cultivated on a solid medium yielded a rate of about 11 per cent. ; when broth enrichment was used the rate for the same swabs was increased to 25·4 per cent. When the area of skin sampled was increased, either by swabbing a greater area of the hand or a number of small areas over the body, the rate was increased to 56 and 70 per cent. respectively in small numbers of subjects. Repeated swabbing of one area increased the total carrier rate from 46 to 82 per cent. of 22 subjects.

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THE FREQUENCIES OF BACTERIAL SUB-TYPES IN A CARRIER COMMUNITY AND THEIR SIGNIFICANCE

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THE epidemic spread of a bacterial species within a community is ideally studied by maintaining a close bacteriological watch upon it, as by periodic sampling of each member of the community. Except in small communities this method is impracticable, and the spread is usually inferred from an increase in carrier rate, which may be observed as a change in the general carrier rate from one period to another or as a local rise in carrier rate compared with that obtaining at the same time in the general community.

The division of species like *Str. pyogenes* and *Str. pneumoniæ* into a number of well defined sub-types has made the study of the epidemic spread of such pathogenic bacteria much more precise than had been hitherto possible. But care must be taken that the evidence obtained from sub-typing in this way is sufficient to bear the weight of the inferences drawn from it. Thus the validity of the argument that, because two or more strains isolated from different persons or places are of the same type, they are likely to be derived from a common source or one from another, depends upon the distribution of the species or sub-type throughout the environment under study. For example, suppose that in a large community 20 per cent. are pneumococcal carriers and that half of these carry a type II pneumococcus. If we then find that 4 of 40 persons in a dormitory are carrying a type II pneumococcus, no inference about the local spread of type II pneumococci is possible, since the gathering in one place of the four carriers of so common a type may be purely fortuitous. On the other hand, if a type occurring only once in every hundred carriers were to be found in 4 of 40 persons, there is a much smaller probability that the occurrence was due to chance. Some measure of the probability of such chance occurrence is required.

The general carrier rate provides an estimate of the probability, p, that a certain species is carried in a certain situation. The probability of finding it in two such situations sampled at random is p^2, in three, p^3, etc. The same argument applies to sub-types of a species, but when the type frequency is known another treatment is possible; the investigation may be limited to persons who are known to be

carriers of the species and the probability determined that the species carried will belong to a given type.

These procedures are valid only if (a) the occurrence of a carrier in a community is assumed to be a chance occurrence and (b) the carrier rates and the type distribution of the strains carried are estimated from a truly representative sample of the community.

With regard to (a), chance effects are usually defined as those due to a multiplicity of small causes acting independently. In any community a bacteriological survey of all the carriers of a species, with subsequent determination of the types within the species, will yield a frequency distribution of types. That is, by sampling a substantial number of the population, we obtain a distribution of types which have frequencies of 1, 2, 3, etc., and a number of untypable organisms which may be temporarily untypable members of known types or as yet undefined new types. The various types cannot be said to occur independently in the strict sense of the word because each must have been acquired from some other source sometime in the lifetime of the animal concerned and some, perhaps, immediately before the time of sampling. In a healthy community, however, it is probable that the majority have been gradually distributed as the result of a large number of transferences occurring at random over a long period of time, producing a relatively stable community of carriers, and the frequencies of the various types will depend on their nature and the average reaction to them on the part of the carrier host. If this assumption is accepted, then any significant increase in the carrier rate for any one type or of a type frequency among carriers may be taken as an indication that the strains in question have been recently derived from a common source or one from another.

With regard to (b) (the reliability of the type distribution as a random sample of the community at large), it is obvious that in repeated type surveys the frequencies for each type will vary, though, as pneumococcal surveys in health and disease have shown (Finland, 1936-37; 1942), common types often remain common and rare types rare. Moreover, with extended surveys, new rare types will probably be discovered. A distribution of types found in a limited sample will therefore be to some extent unrepresentative of the true frequency of each individual type. It will perhaps be more representative of the general frequency distribution of types (irrespective of their identity) which is likely to change less in repeated surveys, since increases in frequency of certain types will be to some extent offset by decreases in frequency of others. As already noted, the distribution will usually contain a number of untypable strains, and with improvements in typing some may be assigned to known types and others to newly recognised types. But neither of these considerations will affect arguments based on typing performed by standard methods throughout a survey.

Suppose a representative type distribution is available, based on the examination of a large number of strains. It is a finite distribution and the frequencies are absolute numbers. In a finite distribution of 50 types, the probability of occurrence of a type with a frequency of one, for example, will be 1/50. But in strict logic we cannot calculate the probability of finding this type twice, for it occurs only once in the distribution and there is no other similar type with which it can form an identical pair.

We must therefore assume that the frequencies are not absolute but proportional, and that in a distribution of 50 types, a frequency of one represents the probability of $1 : 50 = 0 \cdot 02$; that if the population were again sampled, the given type would turn up again once in 50 isolations. Upon this assumption, the probability that 2 consecutive strains isolated at random would belong to this type is $(1 : 50)^2 = 0 \cdot 0004$. (That is, if we regard the known type distribution as a bag containing varying numbers of different coloured balls, we are not picking out one ball at random, retaining it and comparing it with the next ball picked out; we are drawing a ball, noting its colour, replacing it and reshuffling the bag before the next draw.) With the limitations imposed by these assumptions in mind, we can estimate as follows the probability of observing a single type in one or more persons or sites randomly sampled in the community under study.

We are given a distribution of types of N members of the bacterial species, isolated from healthy members of a large community randomly selected to avoid high type frequencies dependent on localised carrier epidemics, and this is assumed to be a true random sample representing the proportional type frequencies in the community at large.

We wish to calculate the probability of observing a duplication, triplication etc., or more generally an m-plication of types in 2, 3, ... m situations sampled at random.

Let t_1 be the number of distinct types each having a frequency (f) of one, t_2 the number with a frequency of two and so on. The probability of observing, say, chance duplication of a type that occurs but once in the distribution is $(1/N)^2$, and of any of t_1 types, each with a frequency of one, it is $t_1 (1/N)^2$. With a type having a frequency of two it is $(2/N)^2$, and if there are t_2 types having this frequency, the probability of duplication of any of these types is $t_2(2/N)^2$. The total probability, p, of chance duplication, irrespective of the actual type duplicated, is therefore

$$t_1(1/N)^2 + t_2(2/N)^2 + t_3(3/N)^2 + \ldots \text{ etc.}$$
$$= \Sigma t(f/N)^2$$
$$\text{i.e. } p = 1/N^2 \Sigma(f^2 t) \qquad . \qquad . \qquad . \qquad . \qquad (1)$$

More generally, the probability (p_m) of a chance m-plication is $1/N^m \Sigma(f^m t)$, where m is the factor of replication to be considered. The formula is dependable when N is reasonably large.

If n replications are observed in a sample, the number expected on a chance basis will be np, which has a standard deviation of $\sqrt{npq}$, where $q = (1-p)$. If p is small compared with q, the frequencies of chance replications conform to a Poisson distribution and the standard deviation is more simply $\sqrt{np}$.

As an example, the accompanying table shows the frequency of distribution of 27 typable strains of *Str. pyogenes* found by Wright

TABLE

Distribution of frequencies of Griffith's types in 27 typable strains of Str. pyogenes *isolated in a surgical ward*

(1) f	(2) t	(3) ft	(4) f^2t $m=2$	(5) f^3t $m=3$	(6) f^4t $m=4$
1	8	8	8	8	8
2	6	12	24	48	96
3	1	3	9	27	81
4	1	4	16	64	256
$\Sigma f^m t$			57	147	441
N		27			
N^m			729	19683	531441
p_m		One in	12·8	134	1205

(1940) in throat swabs of children on admission to a surgical ward and the estimates of p derived from it. Eight types occurred once, 6 twice, 1 three times and 1 four times. From the values of p_m it will be seen that the odds against chance duplication, triplication and quadruplication of types in the ward are respectively 12 to 1, 133 to 1 and 1204 to 1. In an earlier paper (Miles, 1944) these odds were given as 23 to 1, 584 to 1 and over 17,000 to 1, and are incorrect, being calculated by a method which did not treat the values of f as proportional frequencies. The argument that was advanced about the chance quadruplication of *Str. pyogenes* types is nevertheless still valid, since the newly calculated odds against it are as high as 1204 to 1.

The distribution in the table is incomplete in that untypable strains are not entered. Our treatment of the untypable strains will depend on the kind of answer we want from the distribution. So far the calculations have been based on the probabilities that, if a strain is typable, it will belong to a given type. But if we wish to know the probability of observing replication of types among all carriers of the species, then the number of untypable strains must be added to the total in the determination of N. With regard to the untypable strains themselves, neither of the two possible extreme assumptions,

that all are of the same so far undiscovered type or that each represents a distinct type, is likely to be true. But since without further investigation we have no means of deciding, when a pair of untypable strains is found, whether they are identical or not, the only course open to us is to assume that they are all identical. The result will depend on the proportion of untypable strains in the distribution. If it is high, the odds against chance replication is usually lowered. Suppose for example that 15 untypable strains were found when the 27 typable strains in the table were isolated. Considering them as a single type, the figures 15, 1, 15 and 225 must be added to columns (1), (2), (3) and (4) *respectively in the table and the probability of chance duplication*, including duplication of untypable strains, becomes $282/42^2 = 1 : 6.2$ as against $1 : 12.8$ for the typable strains alone. That is, by admitting untypable strains, we have in this case reduced the odds against chance replication; and, if our aim is to demonstrate epidemiologically significant replications of types, we have made the demonstration more stringent by strengthening the indirect statistical evidence against it.

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SHORT ARTICLES

AZOTOLUENE BLADDER TUMOURS IN RATS

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In 1936 Otsuka and Nagao reported that they had induced tumours in the bladder of rats by per oral administration of 2 3' azotoluene All the 13 animals which had been fed on the drug for at least 122 days showed papillomas, generally appearing multi centrically In 7 cases epithelial proliferation was observed deep down in the submucosa In the majority of cases the bladder epithelium showed metaplasia with marked hyperkeratosis According to Yoshida (1935) 4' amino 2 3' azotoluene also produces, together with hepatomas, similar changes in the urinary bladder, though less regularly

In 1941 43 I made control tests with azotoluene (synthesised from m nitro-toluene and o toluidine) The drug was given as a 20 per cent solution in almond oil Each day 0 1 c c was pipetted well down into the pharynx of 37 white rats receiving a satisfactory diet (casein, rice flour, sugar, arachis oil, cod liver oil, yeast, wheat sprouts and salt mixture) After the administration of azotoluene for 126 545 days (average 312 days), the mucous membrane of the bladder was in all cases found to be quite normal, without metaplasia or epithelial proliferation, the bladders being cut in serial section Otsuka and Nagao had used a basic diet of paddy, and it should be remembered that, when the Japanese experiments were repeated in Europe with 4' amino 2 3' azo toluene and 4 dimethylaminoazobenzene (butter yellow), hepatomas were not obtained until the animals were put on a rice diet (Fischer Wasels, 1937 , Heep, 1936 37 , Maisin et al , 1939) Some of the laboratory diets commonly used in Europe prevented the occurrence of liver tumours, but there was no inhibition with the diet of wheat, maize and oats used by Orr (1940) An inhibiting factor was demonstrated in liver, yeast and cod liver oil According to Kensler et al (1941) the combination of casein and riboflavine seems to be effectively inhibitory

A new series of experiments was started in November 1943 with rats on a diet of rice flour to each 98 g of which 2 g of a 10 15 per cent solution of azotoluene in arachis oil had been added (table I) A slice of carrot was also given, as a rule, every other day On this inferior diet 20 animals with an initial average weight of 182 g fell to about 55 per cent of their initial weight during the experimental period, which lasted from 75 to 359 days (average 151 days) Epithelial changes in the bladder appeared in 7 of the 20 animals Of these 7 rats, 3 (dead after 103 117 days) showed metaplasia with hyper keratosis, 3 (dead after 244 271 days) showed marked papillomatosis together with hyperkeratosis, while one (killed after 359 days) showed hyperkeratosis and great epithelial proliferation, though without any real formation of papillomata Thirteen animals, dead after 75 243 days, showed a normal mucous membrane

The original aim of repeating the Japanese experiments with azotoluene was to work out a method of studying the interesting problem of whether

substances that are carcinogenic for the bladder epithelium, act on it through the urine or through the blood. For this purpose a simplified method of transplanting both ureters to the intestine in rats was worked out in 1942. Of about one hundred animals operated upon 18 per cent. survived for more than 100 days (maximum 246 days) on a normal diet. The necessity of keeping the animals on a nutritively inferior diet in order to obtain bladder tumours seemed to invalidate the use of this method for solving the problem in question.

TABLE I

Bladder lesions in rats following the administration of azotoluene

No.	Sex	Duration of experiment in days	Body weight (g.)		Hyperkeratosis	Papilloma	Stones in the bladder
			Initial	Final			
K. 21	♀	159	150	90	—	—	—
K. 22	♀	124	160	130	—	—	—
K. 23	♀	75	175	75	—	—	—
K. 24	♀	136	150	80	—	—	—
K. 25	♀	99	195	95	—	—	—
K. 26	♂	103	185	80	+	—	+
K. 27	♂	107	210	95	+	—	+
K. 28	♂	92	190	100	—	—	+
K. 29	♂	117	175	85	+	—	+
K. 30	♂	93	145	80	—	—	—
K. 31	♀	103	180	100	—	—	—
K. 32	♀	90	180	85	—	—	—
K. 33	♀	99	160	75	—	—	—
K. 34	♀	359	160	135	+	—	+
K. 35	♀	255	180	138	+	+	+
K. 36	♂	243	215	120	—	—	—
K. 37	♂	244	235	125	+	+	—
K. 38	♂	111	180	80	—	—	—
K. 39	♂	271	220	130	+	+	—
K. 40	♂	133	195	95	—	—	—

Instead, a part of the bladder (about ⅓) was transplanted to the liver in the same experimental animals. About 3 weeks after the transplantation the animals were put on the carcinogenic diet (rice, carrots, azotoluene). The advantage of this method was that it enabled us to study simultaneously, in one and the same animal, the possible hæmatogenous effect (on the bladder transplant) and the possible urogenous effect (on the rest of the bladder) (table II). Eighteen animals survived for 53-232 days after the operation and had, when killed, been fed on azotoluene for 40-205 days. Nine of the 18 animals (dead after 40-95 days' administration of azotoluene) showed metaplasia of the epithelium of the bladder with hyperkeratosis, while four animals which had had azotoluene for 80-205 days showed papilloma formation in addition. Only 5 of the 18 animals showed a normal bladder mucosa after having been fed on azotoluene for 55-95 days. Thus the frequency of epithelial changes was greater in these cases than in the animals which had simply been given azotoluene with a rice diet. Both groups showed a high frequency of stones in the urinary bladder (70 and 20 per cent. respectively). The higher frequency in the animals submitted to operation may have been due to the predisposing effect of the local incision. Epithelial changes were observed in five cases (in three of them marked papilloma formation) without concurrent stone formation. Fourteen control animals on a rice diet—no bladder operations, no administration of azotoluene—showed neither epithelial changes nor stone formation after 175-362 days. Stone formation, therefore, evidently plays no prominent role in the

development of the tumours. The portion of the bladder transplanted to the liver was still in existence in 16 of the 18 cases. In all of them, the transplant showed a vital, well-vascularised bladder wall lined with low transitional epithelium and devoid of either metaplasia or papilloma formation. Among these 16 animals with a normal transplanted mucosa there were 3 with papilloma formation and 9 with hyperkeratosis in the rest of the urinary bladder. From

TABLE II

Experiments in which the administration of azotoluene was preceded by transplantation of part of the bladder to the liver

No.	Sex	Duration of experiment in days		Body weight (g.)		Hyper-keratosis	Papilloma	Stones in bladder	Presence of bladder transplant in liver
		from operation	from beginning of azotoluene administration	Initial	Final				
134	♀	148	112	180	90	+	+	+	−
135	♀	132	95	185	95	−	−	−	+
136	♀	82	55	180	102	−	−	−	−
137	♀	232	205	160	100	+	+	−	+
138	♂	66	43	210	115	+	−	+	+
139	♂	105	82	265	130	−	−	−	+
140	♂	78	55	230	145	+	−	+	+
141	♂	104	81	195	105	+	−	+	+
142	♂	91	78	210	115	+	−	+	+
143	♂	121	108	245	110	+	+	+	+
144	♂	53	40	205	120	+	−	+	+
146	♂	86	68	195	95	+	−	+	+
147	♂	98	80	205	110	+	+	+	+
148	♂	94	76	225	105	−	−	−	+
149	♂	87	69	210	95	+	−	+	+
150	♂	104	95	210	100	+	−	+	+
151	♂	73	64	210	100	+	−	+	+
152	♂	88	79	185	95	−	−	+	+

these results it is evident that the changes in the bladder epithelium which occur in rats on a rice diet with administration of azotoluene require direct contact between the urine and the mucosa of the urinary bladder for their development. With this type of tumour the possibility of a hæmatogenous origin for the changes leading to tumour-formation can be discounted.

It seems natural to suppose that azotoluene or some of its metabolic products are to be found in the urine and from there act directly on the vesical epithelium. Investigations pursued in the Medico-chemical Institution here by Docent Birger Ekman seem to indicate that azotoluene only occurs in small amounts in the urine, where various other substances which are being tested at the moment have been observed in considerable amount.

It would seem probable that both o- and m-toluidine occur in the urine provided the animals are on a rice diet. After having been administered for about 100 days to animals on a rice diet, these substances also have given epithelial changes in the mucous membrane of the bladder, in 1/3rd and 2/3rds respectively of the rats examined.

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576 . 8 . 095 . 38 : 576 . 852 . 21

THE INFLUENCE OF CERTAIN ANTAGONISTIC ORGANISMS UPON ACID-FAST BACTERIA

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The treatment of tuberculosis with antagonistic organisms was initiated by Cantani (1885), who employed an organism designated " *B. termo* " and reported favourable results. Vaudremer (1913) used extracts of *Aspergillus fumigatus* upon a group of tuberculous patients with varying degrees of success. Later work on the action of organisms antagonistic to the acid-fast bacilli has been recorded by Schiller (1925, 1927), Barglowski (1938), and Zorzoli (1940), while the negative effect of penicillin upon the tubercle bacillus has been reported by Abraham *et al.* (1941).

It has already been shown (Soltys, 1944) that culture filtrates of *Aspergillus fumigatus* (N.C.T.C. no. 367) possess the power of inhibiting the growth in artificial culture of both saprophytic and pathogenic mycobacteria. The action of certain known antagonistic organisms upon *Myco. tuberculosis* and *Myco. phlei* has been tested and this paper records the results with *Aspergillus fumigatus.* Culture filtrates of this organism, hereafter referred to as " aspergillin ", were more effective in this respect than cultures of *Actinomyces antibioticus* (no. 6124), while completely negative results were obtained with the other organisms used :— *Penicillium notatum* (no. 4222), *Penicillium patulum* (no. 1722), *Aspergillus niger* (nos. 1161, 1214 and 799), *Aspergillus flavus* (nos. 3891 and 4596), *Trichoderma koeningii* (no. 3676), *Actinomyces albus* (no. 3525) and *Bacillus brevis* (no. 2611), all obtained from the National Collection of Type Cultures. Each organism was grown in various media, Czapek-Dox, Raulin Thom's glucose broth, tryptone broth, and the modified Czapek-Dox described below, with similar results.

Preparation of aspergillin

The most suitable medium was found to be a modified Czapek-Dox medium of the following composition :—

Sodium nitrate	.	.	.	.	.	3·00 g.
Potassium di-hydrogen phosphate	.	.	.	1·00 „
Potassium chloride	.	.	.	.	.	0·50 „
Magnesium sulphate	.	.	.	.	.	0·50 „
Ferrous sulphate	.	.	.	.	.	0·01 „

Tryptone	.	.	.	.	.	.	20·00 g.
Brown sugar	`	.	.	.	.	.	40·00 ,,
Glycerol	.	.	.	.	.	.	50·00 ,,
Tap water	.	.	.	.	.	,	1000·00 ml.

The *p*H is adjusted to 7·2.

Spores from a culture on Sabouraud's agar were floated on the surface of the medium in flasks and incubated at 37° C. A dry mycelium completely covers the surface in 2-3 days, and by the 8th-10th day of incubation has become a thick brownish grey felt, the surface of which is not wettable by water. Considerable variation in the *p*H of the medium occurs during growth. During the first three days it falls from 7·2 to 3·5, then rises to 7·0 about the ninth day, and may, on continued incubation, reach 8·2. Aspergillin may be demonstrated in the medium by the 7th day and reaches a maximum concentration by the 15th-20th day. Crude attempts to maintain the *p*H of the medium at 7·0 by the addition of chalk gave irregular results. After 20 days' incubation cultures were drawn through a Seitz EK filter, tested for sterility and examined without further concentration.

Examination of inhibitory power

The inhibitory power of aspergillin was tested upon one strain of *Myco. phlei* no. 54 N.C.T.C., two strains of *Myco. tuberculosis* (human), " DT ", supplied by the Ministry of Agriculture Veterinary Laboratory, Weybridge, and " IM ", isolated from human sputum, one strain of *M. tuberculosis* (bovine) T 51 supplied by Dr J. Young, Cambridge, and two strains of *Myco. tuberculosis* (avian) 485 and 7169 obtained from Weybridge.

Serial dilutions of aspergillin (1 : 25, 1 : 50, 1 : 100 and 1 : 250) were made in 5 per cent. glycerol broth in 2 oz. " medicine flat " bottles. These were then *inoculated with a small amount (1 to 2 mg.) of an actively growing culture of the* organism to be tested and incubated at 37° C. The growth in each dilution was compared with that of a suitable control at intervals up to 4·6 weeks in the case of the mammalian tubercle bacilli and after six days with *Myco. phlei* and *Myco. tuberculosis* (avian).

Results

Myco. tuberculosis, human, bovine or avian, was completely inhibited in a dilution of 1 : 25 and partially in a dilution of 1 : 50. *Myco. phlei* was completely inhibited by aspergillin in a dilution of 1 : 50 and partially in 1 : 100.

Each of the strains of the mycobacteria used was suspended in aspergillin for 21 days at 37° C., then subcultivated on Dorset's egg medium, when growth occurred normally. However, two highly virulent strains of *Myco. tuberculosis*, " IM " and " T. 51 ", subjected to the same procedure and inoculated subcutaneously in a dose of 0·1 mg. into each of four guinea-pigs, did not cause death in six months, while a similar dose of the same strains untreated produced fatal tuberculosis in guinea-pigs within six weeks.

Aspergillin is non-toxic to laboratory animals and is heat-stable; 5·0 c.c. inoculated intravenously into a rabbit and 0·5 c.c. into a mouse every day for seven days were not more toxic than the same dose of medium alone. The inhibitory power of the fluid was not destroyed by boiling for one hour.

Discussion

It has been shown that *Aspergillus fumigatus*, N.C.T.C. strain no. 367, the most active of several strains tested, produces, in a suitable cultural environment, an antibacterial substance " aspergillin ", which interferes with the growth of both pathogenic and saprophytic mycobacteria. The development

of this substance would appear to be dependent to a considerable degree on the composition of the medium in which the organism is grown, and it is conceivable that some of those organisms which as yet have given negative results, might, under suitable conditions of growth, yield antibiotic substances.

Four different antibiotic substances produced by *Aspergillus fumigatus*, two of which are, however, closely related chemically, have already been described (Oxford and Raistrick, 1942 ; Chain *et al.*, 1943 ; Waksman, Horning and Spencer, 1943 ; Menzel, Wintersteiner and Hoogerheide, 1944). Purification of the filtrate now described and the isolation of its active principle will be required to establish the relationship, if any, between it and the other antibiotics produced by *Aspergillus fumigatus*. Recently, Jennings (1945) has suggested that helvolic acid is probably responsible for the antibiotic activity of *Aspergillus fumigatus* which has been demonstrated by various workers. She found that helvolic acid inhibits the growth of the tubercle bacillus (human type), partially at a dilution of 1 : 100,000 and completely at 1 : 10,000.

Summary

Culture filtrates of *Aspergillus fumigatus*, N.C.T.C. no. 367, grown on a modified Czapek-Dox medium containing 2 per cent. tryptone, 4 per cent. brown sugar and 5 per cent. glycerol are antagonistic to the in-vitro growth of *Myco. tuberculosis* (human, bovine and avian types) and *Myco. phlei* ; the filtrates are heat-stable, and non-pathogenic to laboratory animals.

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576 . 8 . 097 . 34 (*Br. abortus*) : 576 . 8 . 077 . 34
+576 . 8 . 077 . 37

THE INCIDENCE OF SERA CONTAINING AGGLUTININS FOR BR. ABORTUS AMONG SAMPLES SUBMITTED FOR THE WASSERMANN REACTION AND THE WIDAL TEST

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In this laboratory two sets of observations on this subject have been recorded. Between April and October 1927 Harrison and Wilson (1928) examined 998 Wassermann sera and 42 Widal sera in which there were no agglutinins for the enteric organisms. Between January 1929 and November 1932 Wade (1933) tested 1000 sera negative in the Widal test.

The object of this note is to record similar observations made on 1392 Wassermann sera submitted during April, May and June 1945 and on 4925 Widal-negative sera tested between January 1933 and May 1945, with a view to comparing results over a considerable number of years.

Technique

The Wassermann sera were diluted with saline and mixed with an equal volume of *Brucella abortus* suspension so as to give final serum dilutions of 1:20, 1:40, 1:80 and 1:100. Suspensions were kindly supplied by the Standards Laboratory, Oxford. The mixtures were incubated for 18 hours at 56° C. in a water-bath and the results read against an illuminated dark background. When agglutination was observed up to the 1:160 dilution the serum was re-tested in higher dilutions. Widal sera were tested in dilutions from 1:40 to 1:2560.

Results with Wassermann sera

These are summarised in table I. The percentage of reactors (agglutination at 1:20 or higher) was 3·6. Harrison and Wilson found 5·4 per cent. of reactors at a titre of 1:10 or higher but at a titre of 1:20 or higher their figure was 3·7. Thus the results of the two series are almost identical and it would appear that there has been no marked change in the incidence of Brucella infection among the population in this region during the intervening period.

The two series were probably sufficiently similar as regards age and sex to make the comparison valid. There was no significant difference as regards age. Harrison and Wilson's series comprised more males than females. They found a higher rate of incidence among females which was just statistically significant, but this included reactors at a 1:10 dilution. It is not possible to say to what extent their 1:10 reactors accounted for their higher female rate. In the present series there was no significant difference in the rates for the two sexes, and the fact that there were twice as many females as males appears to be unimportant.

The distribution of titres is shown in table II. There were a few cases with titres which are unlikely to be met with apart from active infection, but as a rule it was not possible to obtain their histories. In two cases a short history

was secured. The one which had a titre of 1 : 640 was complicated by tuber-
culosis. The other (titre 1 : 2560) was a woman aged 34 years who had aborted,

TABLE I

*Age and sex distribution and incidence of " Wassermann " sera with an
agglutinating titre of 1 : 20 or higher for* Brucella abortus

Age in years	Males			Females			Total		
	No. tested	No. positive	Percentage positive	No. tested	No. positive	Percentage positive	No. tested	No. positive	Percentage positive
0-10	5	0	...	10	0	...	15	0	...
11-20	30	2	6·6	115	2	1·6	145	4	2·7
21-30	83	0	...	393	16	4·0	476	16	3·4
31-40	118	3	2·5	215	13	6·0	333	16	4·8
41-50	90	5	5·5	84	2	2·3	174	7	4·0
51-60	65	1	1·5	47	1	2·1	112	2	1·8
61-70	31	2	6·4	23	1	4·3	54	3	5·9
71 and over	5	1	20·0	3	0	...	8	1	12·5
Unknown	14	0	...	61	2	3·2	75	2	2·6
Total .	441	14	3·1	951	37	3·9	1392	51	3·6

TABLE II

*Titre of agglutinins in routine Wassermann and Widal sera
reacting with* Brucella abortus

Sera submitted for	Number of sera showing titres of								Total number positive	Total number negative
	1 : 20	1 : 40	1 : 80	1 : 160	1 : 320	1 : 640	1 : 1280	1 : 2560		
Wassermann .	19	15	7	6	2	1	0	1	51	1341
Widal . .	0	0	7	10	20	31	23	44	135	4790

She was afebrile and it was not possible to determine with any certainty whether
the abortion was due to Brucella infection. The remaining reactions were
consonant with subclinical infections, a finding which is substantially in agree-
ment with that of Harrison and Wilson.

Results with Widal sera

These sera contained no agglutinins for the following Salmonella organisms—
typhosum, paratyphosum A, B and C, *typhi murium* and *enteritidis.* The results
are set out in table III.

Over the whole period of about 12½ years the sera with agglutinins for
Brucella abortus at a titre of 1 : 20 or higher amounted to 2·8 per cent. The
figure ranged between 0·9 and 6·3 per cent. in different years but in only four
years did it exceed 3 per cent. There was no significant difference between the
sexes, though the number of males was slightly higher. The great majority
of reactors were over 15 years of age ; only 0·24 per cent.were under 15 years.

The distribution of titres (table II) was quite different from that of the
Wassermann sera. Nearly all titres were at the higher dilutions, which would
suggest active clinical infection. Some of these sera were sent from cases
suspected of Brucella infection but the great majority were from previously

undiagnosed cases   The inference is that there is still a considerable number of cases of febrile illness which are detected because a suspicion of *Brucella abortus* is customarily included with routine Widal tests   The number of cases thus detected justifies the continuance of this practice   It is not, however, permissible to deduce from these results anything about the actual incidence of the disease in the population

TABLE III

Number of "Widal" sera which agglutinated Brucella abortus *at a titre of 1 20 or higher for each of the years 1933 1945*

Year	No of sera examined	No positive	Percentage positive	No under 15 years
1933	390	10*	2 5	0
1934	353	3	0 9	0
1935	342	10	2 9	0
1936	385	10	2 5	0
1937	429	12	2 6	1
1938	446	19	4 3	1
1939	539	10	1 8	1
1940	743	10	1 3	3
1941	551	12	2 1	1
1942	286	18	6 3	2
1943	255	12	4 8	1
1944	113	7	3 2	2
1945 (May)	83	2	2 4	0
1933 45	4925	135†	2 8	12

* Age and sex of one unknown
† 71 males 63 females

Wade's series, which immediately preceded the present series, gave a similar result   Over a period of four years 1066 sera were tested of which 28 were positive (2 6 per cent)   Dilutions from 1 40 were tested but the reacting sera had high titres, 14 of 1 2560, 10 of 1 1280, 3 of 1 640 and 1 of 1 320   In the much smaller series of Harrison and Wilson 11 of 42 sera reacted at 1 10 or higher, 5 of them at 1 160 to 1 1280

Summary

In the three series of observations from this laboratory covering the years from 1927 to 1945, the investigation of Wassermann sera for Brucella agglutinins revealed about 3 6 to 3 7 per cent of positives at a dilution of 1 20 or higher The majority of these had titres which suggest sub clinical infection but a few had high titres such as are rarely seen apart from active disease   The incidence has not changed during the period of eighteen years covered by the two sets of observations

Widal sera which contained no agglutinins for enteric organisms yielded a varying percentage of reactors from year to year, which ranged between 0 9 and 6 3   The average for 1929 32 was 2 6 per cent , for 1933 45 2 8 per cent The majority of reacting sera had titres which suggested active infection   It would appear that there has been no marked change in the incidence of Brucella infections in this area during the sixteen years covered by the observations

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AN INTRAPERICARDIAL TERATOMA IN AN INFANT

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(PLATES XXXV AND XXXVI)

Case report

History. D. W., a male infant 10 weeks old, was admitted to the Alfred Hospital, Melbourne, in October 1942 with a history of choking, difficulty of swallowing and loss of weight for two weeks. He weighed 6 lb. 13 oz. at birth and had had a poor appetite since birth. The only other child was a girl aged 3 who had congenital deafness. Examination showed an ill-nourished infant with a large area dull to percussion over almost the whole anterior aspect of the chest, and radiograms showed a greatly enlarged pericardial shadow with both lungs compressed behind it. Clear yellow fluid was obtained by puncture of the pericardium, and many ounces were drawn off by repeated aspiration during ensuing weeks, with reduction in size of the X-ray shadow and improvement in the child's condition. The fluid contained only a few red corpuscles and lymphocytes, was sterile on culture and injection into a guinea-pig failed to produce tuberculosis. The electro-cardiogram was normal; repeated Wassermann tests were negative. He gradually gained weight and strength, was able to sit up in bed and play actively without distress and was allowed to return home. He was readmitted in April 1943 with return of the former symptoms and radiograms showed great re-accumulation of the pericardial fluid, 6 oz. of which were aspirated with relief of symptoms. On 13th May the child, who had shown no previous signs of distress, died suddenly while being washed.

Necropsy showed a rather thin child with distension of the thorax and abdomen due to great enlargement of the pericardial cavity, which measured 12 cm. in transverse diameter and had compressed the lungs posteriorly against the chest wall. No abnormalities were found in any thoracic or abdominal organs other than the heart and lungs.

When the thin parietal pericardium was opened, many ounces of clear yellow fluid escaped and a well defined ovoid lobulated cystic tumour, 8·5 × 6·5 × 6 cm., was seen projecting from the right side of the base of the heart. The heart was displaced to the left and rotated so that, viewed from the front, only the right ventricle was visible (fig. 1). The right atrium was compressed behind the upper half of the tumour, to which it adhered slightly by easily separable secondary adhesions. The attachment proper of the tumour was by a pedicle 2 cm. in diameter uniting it to the right and posterior aspects of the ascending part of the aortic arch and to the tissues of the atrio-ventricular sulcus to the right of the roots of the aorta and pulmonary artery. The blood supply of the tumour consisted of several small vessels coming from those of the adventitia of the aorta and distributed mainly over the anterior aspect of the tumour. Careful dissection failed to show any branches to the tumour from the contiguous right coronary artery.

After fixation, the anterior part of the tumour was sliced off by a vertical frontal cut, showing it to consist of a collection of cysts measuring up to 5 cm. in diameter and containing gelatinous fluid (fig. 2). Windows cut in the anterior

INTRAPERICARDIAL TERATOMA

FIG 1—Photograph of the teratoma (T) and heart (H) after reflexion of the parietal pericardium   A = aorta, P = pulmonary artery, L = lungs

INTRAPEPICARDIAL TERATOMA
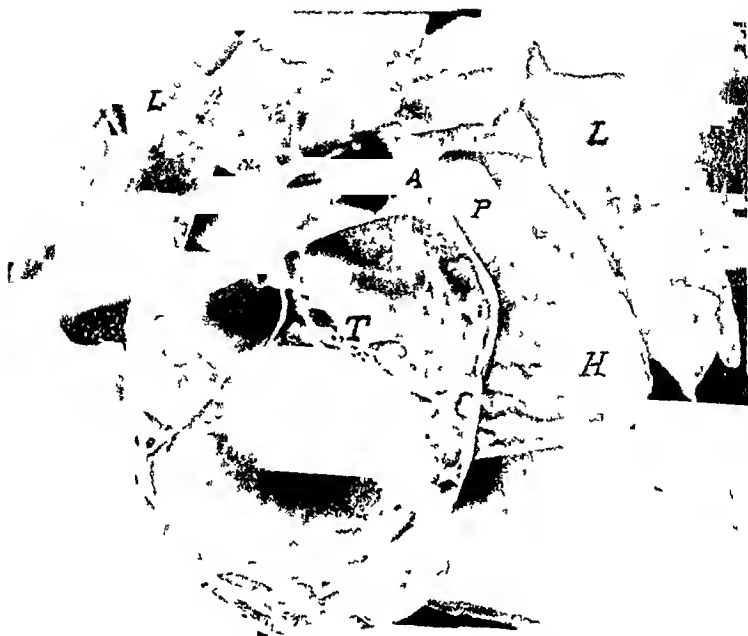
Fig 2 —The front portion of the tumour has been sliced off and the pericardium has been trimmed away to display the compressed lungs Note the close fusion of the wall of the tumour with the aorta

Fig 3 —Gastric mucosa lining a cyst in the teratoma   ×120

Fig 4 —Pancreatic tissue with islets of Langerhans   ×120

walls of the aorta and pulmonary artery revealed the following features The wall of the aorta was pushed inwards by the growth, so that a horizontal cross section of its lumen was semicircular The indented part of the aortic wall was thinner than elsewhere (0 9 instead of 2 mm) and the thinned wall adhered closely to the wall of the uppermost cyst of the tumour, so that together they formed a partition only 1 mm thick separating the aortic lumen from the cyst cavity The aortic cusps were normal The pulmonary artery was much enlarged, both in calibre and in the thickness of its wall, its internal diameter being 1 cm and its wall 2 mm thick The pulmonary orifice and cusps were normal and there was no patency of the ductus arteriosus The wall of the right ventricle was abnormally thick

Histological examination The anterior sliced off part of the tumour was cut into 20 blocks, from all of which micro sections were prepared Most of the cysts were lined by ciliated pseudo stratified *respiratory epithelium* , others were lined by *gastric mucosa* with typical gastric glands containing distinct parietal and chief cells (fig 3) A few cysts were lined by simple *goblet celled epithelium* of intestinal type Small scattered areas of *pancreatic tissue* with islets of Langerhans were present (fig 4) *Mucous and mixed glands* accompanied some of the respiratory cysts and there were a few foci of typical *salivary gland tissue* with ducts *Stratified squamous epithelium* was scanty , it was always in continuity with respiratory epithelium and nowhere did it show epidermal characters *Neuroglial tissue*, devoid of nerve cells, formed small scattered patches or long strips in the cyst walls, and there were a few small groups of slightly pigmented nerve cells like those of the *sympathetic ganglia Striated skeletal muscle fibres* were plentiful around the periphery of the tumour Small nodules of *cartilage* and occasional tiny foci of *bone* with *hæmopoietic marrow* were present, the former usually close to respiratory cysts *Non striated muscle* was abundant, arranged around respiratory cysts or forming a muscularis mucosæ to gastric cysts

Comment

The tumour is a benign congenital polycystic teratoma, containing many varieties of tissue, the most plentiful of which is respiratory tract tissue It had arisen in close connection with the root of the aorta and had grown into the pericardial cavity

I have been able to discover only four previous records of intrapericardial teratoma Joel (1890) described a cystic teratoma the size of a hen's egg attached to the adventitia of the pulmonary artery in a boy of 14 Grimm (1927) briefly reported a large polycystic teratoma which enveloped the heart and was attached to the adventitia of the great vessels in an infant of 3 months Jellen and Fisher (1936) saw a polycystic teratoma 5 cm in diameter attached to the aorta and base of the heart in an infant 4 weeks old Gebauer (1942 43) surgically removed a large intrapericardial teratoma from a girl aged 10 years , its close attachment to the root of the aorta led to surgical injury of this vessel and fatal hæmorrhage

In all 5 cases the position of the tumour was closely alike, the main attachment being near the root of the great vessels, especially the aorta This suggests that intrapericardial teratoma is only a peculiarly situated variety of mediastinal teratoma, a tumour situated most often in the upper part of the anterior mediastinum, i e at a level nearly corresponding to that of the base of the heart Whether a tumour arising in this region shall assume an anterior mediastinal or an intrapericardial position may depend only on the direction in which its main growth takes place Closer study of the primary attachments and blood supply of the anterior mediastinal teratomas is desirable It may be that a restricted area around the root of the great vessels, corresponding to the usual attachment of the intrapericardial variety, is the primary nidus of all the teratomas of this region

All 5 reported intrapericardial teratomas have been in infants or young children and there is no doubt that they were all already well advanced at birth. Mediastinal teratomas, however, often do not produce symptoms until adult life. This difference is not surprising, since a tumour closely adherent to the great vessels and occupying the pericardium along with the heart might be expected to produce serious results earlier than one growing in the anterior mediastinum. Added to the embarrassment caused by the tumour itself, there might also be that of a large pericardial effusion, as in the present case.

The hypertrophied state of the pulmonary artery and right ventricle in the present case is of interest. Since no cardiac malformation or patent ductus arteriosus was present, this must be attributed to obstruction of the pulmonary circulation due to long-standing compression of the lungs by the large pericardial effusion. The infant's death presumably resulted from sudden cardiac failure in some way related to the displacement of the heart by the tumour, the pericardial effusion and the pulmonary compression and circulatory obstruction.

Summary

A large intrapericardial teratoma attached to the aortic trunk in an infant is described ; four other reported cases are referred to.

For the clinical notes of the case I am indebted to Dr Laurence Stokes of the Alfred Hospital, Melbourne. The photographs were made by my wife and the photomicrographs by Mr F. Watson.

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SARCOMA OF THE DIAPHRAGM WITH AN INTRA-AORTIC METASTASIS

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(PLATES XXXVII-XXXIX)

CASE REPORT

Clinical history

A man aged 42 was admitted to hospital complaining of pain in the right hypochondrium, right shoulder and back of the neck. Three years before, he had developed a cough which was not productive of sputum but which worried him a good deal, and he was discharged from the Army with a diagnosis of chronic bronchitis, although no evidence of any bronchial affection was shown on X-ray examination.

SARCOMA OF DIAPHRAGM

Fig 1 —Vertical (antero posterior) section of liver and diaphragm   This shows the diaphragm grossly thickened by tumour, which has extended downwards over the anterior surface of the liver and has undergone central necrosis and cavitation The tumour has compressed but not invaded the liver

Four months before admission he noticed a dull, aching pain in the right hypochondrium The pain was constant, did not radiate, was aggravated by coughing and had not changed its character since its onset

Three months before admission he developed an additional, less constant pain in the region of the cervical spine This sometimes radiated to the right shoulder and was burning in character Recently he had noticed a little breathlessness on exertion, and there had, he thought, been some slight loss of weight

On examination the patient was pale but well nourished Early clubbing of the fingers was present There was a fulness in the right hypochrondrium and palpation revealed a firm mass which moved on respiration and seemed to be in contiguity with the liver Dull to percussion, its lower limits could not be clearly defined, the mass falling away from the abdominal wall about four inches below the costal margin It was tender and some muscular rigidity prevented closer examination

The most outstanding physical sign noted in the chest was diminished movement at the right base, with poor air entry as compared with the left base There were also a few moist râles on inspiration No other clinical evidence of lung disease was elicited, and the other systems were normal on physical examination

X ray examination of the lungs showed rounded opacities at both bases, the appearances being those of metastases Renal carcinoma was suggested

Blood examination showed Hb 75 per cent , red cells 4 million, and white cells 14,200, of which 68 per cent were polymorphonuclears, 28 per cent lymphocytes and 4 per cent monocytes

Course

The pains of which the patient complained did not alter in character, but became very severe and were only slightly relieved by morphia Bouts of low grade temperature were observed It was thought that the mass in the right hypochondrium was due to secondary involvement of the liver and unsuccessful attempts were made to find the primary growth A pleural effusion developed on the right side Hæmorrhagic fluid which was aspirated was found to contain large polyhedral undifferentiated cells These were regarded as probably endothelial in origin

A little later the patient's cough became more productive, and he began to produce blood stained sputum One such sample contained fragments of white tissue This tissue was found on examination to be composed of a mass of densely packed, round, neoplastic cells, showing an occasional mitotic figure The cells were somewhat degenerated and the features suggested an un differentiated carcinoma or round cell sarcoma

Five weeks after admission to hospital the patient suddenly collapsed and died

Post mortem findings

The liver (fig 1) was enlarged and weighed 3010 g Over its right anterior surface was a large cystic swelling which had compressed but not invaded the liver tissue The cystic swelling was attached to the peritoneum of the lateral abdominal wall On further dissection it was found that the cystic swelling was adherent to the right dome of the diaphragm, which was thickened, and in turn very adherent to the eighth and ninth ribs in the mid axillary line

On section there was a large fleshy mass growing from the diaphragm The mass extended posteriorly (infiltrating the muscle), laterally (involving the ribs) and downwards and anteriorly over the right lobe of the liver Part of it had undergone central necrosis, with formation of the cyst previously mentioned, which contained blood and necrotic debris

Numerous white or cream coloured nodules of secondary growth were scattered throughout both lungs ; one of these nodules was in close proximity to a small bronchus. A soft fleshy tumour nodule was adherent to a coil of jejunum.

There was little of interest elsewhere, except in the cardiovascular system, where a pedunculated nodule about the size of a pea was found growing from near the commencement of the root of the aorta into the lumen. The base of the nodule was related to two small plaques of atheroma : on section it contained soft pultaceous material. The media and adventitia underlying the nodule appeared healthy.

No other metastases were found, and the lymphatic system was not involved.

Histology

Sections of the primary tumour stained with hæmatoxylin and eosin presented a very cellular and pleomorphic appearance (fig. 2). Small round and spheroidal cells predominated, others were larger, while some were spindle-shaped and arranged in whorls. Many multinucleated giant cells were present and many of the cells showed various phases of mitosis. The stroma was very scanty : muscle fibres from the diaphragm were present in one section. Sections stained with phosphotungstic acid-hæmatoxylin did not show any striations and sections stained with iron hæmatoxylin failed to show any of the so-called spider-cells. In the absence of these differentiating features to support a diagnosis of myosarcoma, the non-committal diagnosis of mixed cell sarcoma was made.

In sections of lung similar tumour tissue was found (fig. 3). It was seen invading the bronchial wall and tumour cells were present in the lumen of the bronchus (fig. 3). Another part of the section showed tumour cells in the lumen of a blood vessel (fig. 4).

The aortic nodule (fig. 5) was found to be related to a small patch of thrombus on an atheromatous intimal plaque. It contained cells similar to those of the primary growth, some of them showing mitosis. The media and adventitia were healthy.

Diagnosis

Mixed cell sarcoma of the diaphragm with metastasis to the lungs, abdomen and intima of the aorta.

Discussion

The correct diagnosis of this case was not made during life, probably because a primary diaphragmatic tumour was not considered. When the history is reviewed in the light of the post-mortem findings it is felt that it does afford an indication of the diagnosis.

The man suddenly developed a persistent cough, bad enough for him to be discharged from the army, but without any clinical or radiological evidence of a lung lesion to explain it. Such a chronic, non-productive cough is, of course, very suggestive of a bronchial carcinoma. The pain of which he complained, however, had the typical distribution of diaphragmatic pain. Later, when secondaries had developed in the lung, they were distributed equally in the basal region of both sides, yet the physical sign of diminished movement was only present on the right side. Some slight distortion of the right dome of the diaphragm had been noticed on the X-ray film, but this was not thought to be outwith normal limits and the clue thus presented was not followed up.

Pathologically the case is of interest for several reasons. 1. The site of the tumour is unusual. It appears to arise from the diaphragm and the general characters of the cells suggest a myomatous origin, though no positive histological evidence of this could be found.

SARCOMA OF DIAPHRAGM
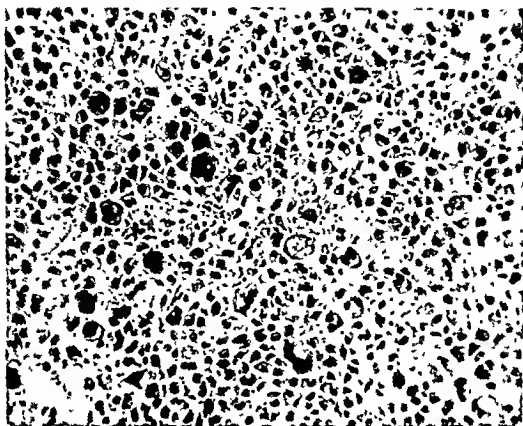
FIG. 2.—Microscopic appearance of the primary tumour, which is very cellular and pleomorphic. ×220.
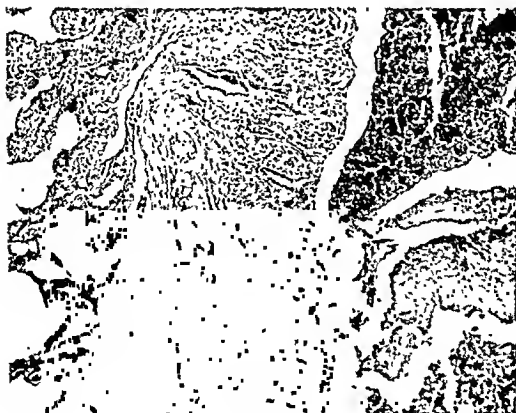
FIG. 3.—Tumour cells (right) in lumen of bronchus. ×50.

SARCOMA OF DIAPHRAGM

Fig 4—Nodule of tumour attached to ...a of a blood
vessel in the lung   × 75

Fig 5—Low power view of the aorta, shewing the intima invaded by tumour
in relation to an atheromatous plaque   × 50

2. During life, tumour cells were recovered from the sputum. These had a close resemblance to those of the primary growth, and, *post mortem*, there was bronchial invasion, with similar cells in the lumen.

3. Metastasis was present in the aorta. It seems not unlikely that tumour cells in the blood stream were enmeshed by the small plaque of atheroma present and there continued to grow.

We wish to thank Professor Murray Lyon for his permission to publish this case report. We also wish to thank Professor A. M. Drennan and Professor D. F. Cappell for their help in the compilation of this paper. We are indebted to Mr T. C. Dodds for the illustrations.

DISSECTING ANEURYSM OF THE AORTA ASSOCIATED WITH INTRACRANIAL ANEURYSM AND CEREBRAL HÆMORRHAGE

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The case described is worthy of record even if the conditions found were merely coincidental. Certain considerations, however, suggest that they were related.

Case history

W. H., aged 69, died at 2 a.m. on 10.11.42. The wife's statement was "... I have been married 46 years. My husband was a platelayer on the railway and has enjoyed fairly good health except that he occasionally suffered from stomach trouble due to a rupture he sustained at work. He has not had a doctor to see him since 1918 when he had pneumonia during the 'flu epidemic. He stopped working on the railway four years ago owing to his age. He still pottered about the garden and did odd jobs about the house. On Thursday 5th November he complained about his stomach and back and I thought he had a touch of 'flu. He went to bed early, about 8 p.m. He stopped in bed on Friday but got up on Saturday and appeared to be better. He also got up on Sunday and Monday. On Monday evening he had some milk sops and went to bed about 9.30 p.m. He appeared to be all right and in his usual spirits. I followed him to bed about 10 p.m. About 1.50 a.m. he got up out of bed and said he was going to the lavatory at the end of the landing. A few minutes later I heard a groan and found him lying on the floor of the lavatory. He appeared to be dead. A doctor who was called immediately found that he was dead".

Post-mortem findings

Autopsy was performed 60 hours after death. A thick-set fat man with a huge left scrotal hernia.

Cranial cavity. Brain 46 oz. Dura mater thickened, pia mater slightly opaque but about normal for his age. There was some bulging of the commencement of the right anterior cerebral artery and slight aneurysmal dilation at its junction with the anterior communicating artery. On the left middle cerebral artery near its origin there was a large dumb-bell shaped or bilobed aneurysm. The larger sac was about 1 cm., the smaller about 0·5 cm. in diameter. Otherwise, apart from a few small atheromatous patches, the cerebral arteries appeared healthy.

On section of the cerebrum a recent hæmorrhage approximately 1¼ inches in diameter was discovered in the typical claustro-putamen region on the left side. Despite careful search it was not possible to demonstrate that the hæmorrhage had arisen from the rupture of an aneurysmal condition of a neighbouring artery (cf. Globus, 1943).

Thoracic cavity. There was a considerable degree of hypertrophy and dilation of the left ventricle of the heart. The valves appeared normal. The coronary arteries were patent, wide and thick walled; atheroma negligible. The first part of the aorta was dilated. A T-shaped split was found in the intima, the long arm commencing at a point between the left common carotid and subclavian arteries and running to the left for a distance of 2 inches, while the smaller tear, about 1 inch long, was situated at right angles to it. A large dissecting aneurysm extended from the base of the heart to the commencement of the common iliac arteries. The sac was largest over the region of the arch and there was a considerable quantity of blood between the layers from the base of the heart to the termination of the arch. In the descending aorta the intima was stripped from the other layers and lay loose in the lumen. Approximately half the circumference of this part of the aorta had been dissected, the remaining half being intact; no clot was seen. There was a little blood clot external to the stripped intima at the commencement of the right common carotid artery, and to a lesser extent in the left common iliac artery and around the bifurcation. There was slight, almost negligible atheroma of the aorta.

The left pleural cavity was full of blood clot which weighed 3 lb., the secondary rupture of the aorta having occurred in the neighbourhood of the root of the left lung. The left lung was collapsed; the right, which showed basal congestion and apical œdema, was tough and emphysematous.

Abdominal cavity. Stomach empty. No scars in stomach or duodenum. The liver showed slight chronic venous congestion. No gross abnormality of spleen, kidneys, urinary bladder or prostate.

Microscopic findings

Section through the primary tear in the aorta showed that the main mass of blood lay between the adventitia and the media. Immediately opposite the tear the media was also infiltrated by blood cells and its outer portion was considerably disorganised. No muscular "fault" was seen and the elastic pattern was well defined. There was considerable fibroblastic activity in the adventitia, probably explained by the infiltration of red cells. The intima appeared normal. Section through the intracranial aneurysm showed that the wall of the sac was composed entirely of poorly cellular fibrous tissue. The vessel from which the aneurysm arose showed normal muscle and elastic tissue, but there was a considerable degree of atheromatous degeneration, which, however, did not seem to extend into the aneurysm. Mucicarmine staining showed small quantities of mucin in the media of the aorta but none in the left middle cerebral artery.

Discussion

It is probable that the primary or internal tear in the aorta occurred on 5th November, and that the symptoms at that time were caused by the intramural hæmorrhage. The subsequent general improvement in the patient's condition is quite consistent with dissection, and the sudden death corresponds with the massive hæmorrhage into the pleural cavity from the secondary or external tear.

Cerebral aneurysm is an uncommon concomitant in dissecting aneurysm. As far as we are aware only one other case has been reported. Beadles (1907-08) quotes Hutchinson's "well known case" of an aneurysmal tumour of the left

internal carotid artery, diagnosed during life (a bruit was heard over the skull), in which death was due to a dissecting aneurysm of the abdominal aorta It is the only occasion on which it has been noted by one of us (D G) in a consecutive series of over 100 cases of dissecting aneurysm

It is unlikely that the recent cerebral hæmorrhage was fortuitous The pathological changes suggested that the cerebral bleeding commenced at the time of the aortic dissection and that a sudden rise in blood pressure in a hypertensive subject was the immediate exciting cause of both lesions

In the attempt to find a predisposing cause to explain the apparently unrelated lesions, cerebral aneurysm and dissecting aneurysm, we must admit the argument to be speculative In a patient aged 69 atherosclerosis would be the first consideration The dilation of the right anterior cerebral artery and the atheromatous patch immediately adjacent to the wall of the cerebral aneurysm indicate degenerative changes Some authorities stress the occurrence of focal arteriosclerosis confined to the region of intracranial aneurysms (Schmidt, 1930 31, Strauss *et al*, 1932) Others argue that arteriosclerosis even when present is not necessarily the primary cause, and deny that intimal changes if present may be secondary to a weakened media (*vide infra*) Moreover, it has been pointed out that the aneurysmal wall may show obvious arteriosclerotic changes whilst the rest of the cerebral vessels are quite free microscopically (Mitchell and Angrist, 1943) In our case the aorta was remarkably free from atheroma and the cerebral vessels were only slightly affected That atheroma may be a factor in dissection is undisputed, the dissecting process commencing at or near the edge of a rigid plaque and not usually through the base of the ulcer (Shennan, 1934, Logue, 1943), but even here the trend of opinion is that medial degenerative changes are more fundamental

Embolism needs consideration, since mycotic aneurysms are a distinct entity, though aortic dissection is rarely attributable to this cause Endocarditis was not present, nor was there any supporting clinical or pathological evidence of embolism

Syphilis was unlikely, since this disease plays little or no part in either condition Moreover syphilitic aortitis was absent

It has been postulated that frequently a patchy degeneration of the tunica media is the most important determining factor in dissecting aneurysm Gsell (1928), Erdheim (1930), Cellina (1931), Shennan (1934) and others have made notable contributions to our knowledge Rottino's recent detailed study of 12 cases (1939) confirms Gsell's contention that the lesions occur in the ascending aorta and arch He states that they may progress by stages from simple muscle loss to eventual cyst formation ~The ætiology of these non inflammatory lesions is obscure Toxins and epinephrine are amongst the agents which have been incriminated (Sailer, 1942) Leary and Weiss (1940) have produced medio necrosis in rabbits by massive doses of vitamin D or by repeated dosage with epinephrine Dissection may occur with epinephrine but not with vitamin D, possibly because of the severe paroxysmal hypertension associated with it The same authors describe the induction of atherosclerosis in a rabbit by cholesterol feeding, the cholesterol was stopped and some time later the animal died suddenly from a dissecting aneurysm

In intracranial aneurysms local defects of the middle coat occurring at the angle of branching of certain cerebral vessels have been described (Turnbull, 1914 15, Forbus, 1930, Bremner, 1943) However, developmental faults occur frequently without sign of aneurysm, and a further unknown factor, ' possibly toxic or metabolic ", has been incriminated (Richardson and Hyland, 1941) Although the latter is conjectural, a case has been reported in which an acute inflammatory reaction was found in the neck of the aneurysm (Mitchell and Angrist) Other authorities discredit medial developmental defects, while Glynn (1940) stresses the importance of the peculiar distribution of elastic tissue

Examination of the specimen after removal revealed a firm adhesion to the bladder wall over a rounded area approximately 2 inches in diameter. In the centre of this area there were small rounded red excrescences on the mucosal surface. A section through the growth demonstrated that it was a multilocular cystic tumour resembling those found in the ovary (fig. 1). It appeared to be completely encapsulated except at its attachment to the bladder wall, where it seemed to invade the mucosa.

Microscopic appearance

A section of the bladder adjacent to the area of apparent neoplastic invasion shows that the usual stratified transitional epithelium is replaced by mucus-secreting cells (fig. 2). For a short distance the stratified epithelium is interrupted at irregular intervals by isolated mucus-secreting tubular glands. Beyond this the mucosa rises up in the form of a slender papilla covered by stratified transitional epithelium except on the lower part of the distal side, where it becomes thinned to a single layer of flattened cells and then there is a complete and abrupt change to mucus-secreting epithelium. This dips down in the form of tubular glands ending in follicles in which the epithelium shows a tendency to form papillæ. In this way it forms the whole mucosal surface of the area of apparent invasion. The glands open freely on the surface and there is no evidence of obstruction at this level. The surface epithelium is desquamated in a few places and there is diffuse infiltration with lymphocytes and plasma cells of the stroma surrounding the glands.

Deep to this mucous membrane a cystadenoma has formed, the cysts of which are filled with mucoid material and some are lined by clear columnar cells similar to those in the superficial glands. In others the epithelium is of low-grade type, consisting of flattened cells. For the most part the epithelium has undergone degenerative changes and much of it has been shed. The resulting appearance is suggestive of mucoid carcinoma, but the stroma is healthy, the cystic spaces are of comparatively regular shape and there are no signs of active proliferation of the epithelial cells.

In the deepest part of some of the superficial glands ulceration has occurred with formation of granulation tissue. This has led to obstruction and the expansion of the distal portions into small cysts with mucoid contents and flattened epithelium (fig. 3). Similar cysts are seen in close proximity to the tubules in all parts of the glandular mucosa, but there is no sign of infiltration of the mucosa by the cystadenoma. The remainder of the tumour is completely encapsulated and there is no evidence of extension outside the limits of the capsule.

Commentary

Adenomata of the urinary bladder are most commonly found around the trigone and neck and are usually described as small, flat or pedunculated masses projecting into the bladder cavity. They are said to be related to the condition known as cystitis cystica (Cahen, 1888 ; Bayer, 1909). Wilson (1934), in a comprehensive survey of cystitis cystica, came to the conclusion that the cysts were formed by inflammatory obstruction of lacunæ in the trigone or by central degeneration of epithelial inclusions in other parts of the bladder wall.

The present tumour differs from those previously described in certain points. It arises from the fundus and projects backwards beneath the peritoneum. There is an apparent history of long-standing cystitis but there are no signs of epithelial inclusions. There is a gradual thinning of the normal stratified transitional epithelium to a single layer and then an abrupt transition to columnar mucus-secreting epithelium, with tubular glands. These occasionally end in follicles resembling the glands of the prostate. Glandular epithelium is

PLATE XL

CYSTADENOMA OF URINARY BLADDER
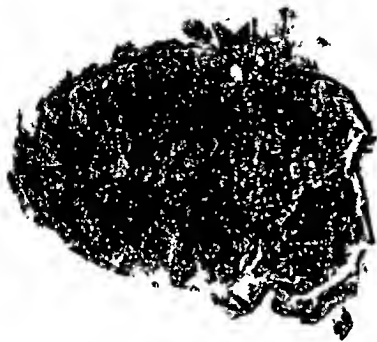
FIG. 1.—Antero-posterior section of the bladder tumour showing the very fine multilocular structure and apparent invasion of the bladder wall. The *portion of bladder wall forms a "coronet"* in the figure. ×⅔ (approx.).

FIG. 2.—Section taken at the edge of the glandular mucosa, showing the thinning of the transitional epithelium and then its replacement by columnar cells. H. and E. ×50.

FIG 3—Section of the basal portions of the tubular glands and upper part of the cystadenoma, showing the tendency to cystic change in many of the glands. In one instance there is a small ulcer in the terminal portion of a gland just above the centrally placed cyst. H. and E. ×17

occasionally found in the bladder at the base of lacunæ but these structures are usually confined to the trigono and neck of the bladder (Albarran, 1892)   No direct connection can be traced between these glands and the cystadenoma, but some of the tubules show changes suggesting that the cysts are derived in the first instance from the distal portions of the tubules

It is suggested that the superficial glandular mucosa is a heterotopic focus of mucus secreting epithelium of prostatic origin which has undergone repeated inflammatory attacks and in the process of healing the basal portions of some of the tubules have become separated off by granulation tissue, subsequently giving rise to a cystadenoma

Summary

A case of solitary cystadenoma of the fundus of the bladder is described The histological appearances suggest that it has arisen from a heterotopic focus of prostatic glandular tissue

I wish to thank Mr T Baron Rose Birmingham United Hospitals, for permission to publish this case

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TUBERCULOSIS IN AN ADENOLYMPHOMA OF THE PAROTID GLAND

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(PLATE XLI)

Adenolymphoma is a rare but clearly defined tumour of the salivary glands It has been reported under a variety of names, the commonest alternative to adenolymphoma being papillary adenocystoma (or cystadenoma) lymphomatosum (Ewing, 1940)   Carmichael, Davie, and Stewart (1935) described 8 cases and gave a comprehensive review of the subject   More recently Berner (1942) in a search of the literature found 63 published cases and added 4 of his own and Lederer and Grayzel (1942) have also reported 4 cases   Martin and Ehrlich (1944) collected no fewer than 22 cases from the records of the Memorial Hospital, New York, covering the period from January 1932 to March 1944 inclusive   They constituted 10 per cent of all benign parotid tumours and 6 per cent of all parotid tumours   In none of these 93 cases was tuberculosis of the lymphoid stroma described   It is the purpose of the present paper to record such an occurrence

Histologically these tumours are composed of a lymphoid stroma in which lie tubules and cysts lined by highly characteristic epithelium   This epithelium

consists of strongly eosinophilic tall columnar cells, arranged in palisade formation, with their nuclei in an even row towards the free margin. At intervals between these cells are basal cells with their nuclei lying close to the basement membrane. The cysts generally contain granular eosinophilic debris, occasionally colloid-like material. Sometimes the tumours show an intracystic papillary structure. The stroma has the characters of normal lymphoid tissue, with germ centres. The tumours are always encapsulated, and show close topographical relationship to a salivary gland.

It is not intended here to discuss again the question of the histogenesis of these tumours. Suffice it to say that the most plausible theory seems to be that they arise from heterotopic salivary gland or salivary duct tissue in a lymph-gland, abnormally blended during development (Nicholson, 1922, 1923).

Case report

Mrs C. H., age 60, complained of a swelling beneath the angle of the right mandible for 2 years, increasing more rapidly in size for 8 or 9 months and particularly during the last 3 weeks. The size had varied a little from day to day. About 15 years previously a sudden swelling, which the patient described as " like a balloon ", had occurred in the same place. It went away quickly, and was neither painful nor tender. At operation, the tumour was stripped fairly easily from the surrounding structures, but in doing so it burst, and " pus " of creamy consistency escaped.

The specimen consisted of an ovoid tumour $5 \times 2.5 \times 2.5$ cm. in size. The cut surface showed numerous small cysts 1·2 mm. in diameter, and towards one pole a larger irregular cyst, 3×4 mm., with a shaggy wall.

On histological examination the tumour is clearly seen to be an adeno-lymphoma (fig. 1), the stroma of which shows the typical appearances of tuberculosis. Although attempts to demonstrate acid-fast bacilli were unsuccessful, the occurrence of miliary tubercles (fig. 2) and of granulomatous and caseous lesions (fig. 3) leaves no reasonable doubt as to the diagnosis. The tumour has a well marked fibrous capsule, which in one area shows atrophied salivary gland acini.

The patient was examined at a later date for evidence of tuberculosis elsewhere, but none was found.

Comment

Carmichael *et al.* conclude that both the epithelial and lymphoid elements participate in the neoplastic process. The present case is of interest in that the lymphoid element reacted to tuberculous infection in the same manner as a normal lymph gland. Probably the rapid growth of the tumour reported by the patient during the last few weeks before operation was due to the secondary tuberculous infection. Worthy of note is the patient's story of a swelling at the same site 15 years previously. This would appear to suggest some abnormality of structure in the salivary gland which might predispose to the later development of the adenolymphoma.

Summary

A typical case of adenolymphoma of the parotid gland with tuberculosis in the lymphoid stroma is reported.

I wish to express my thanks to Mr A. York Mason for the clinical notes and to Dr A. B. Bratton for the photomicrographs.

TUBERCULOSIS IN AN ADENOLYMPHOMA
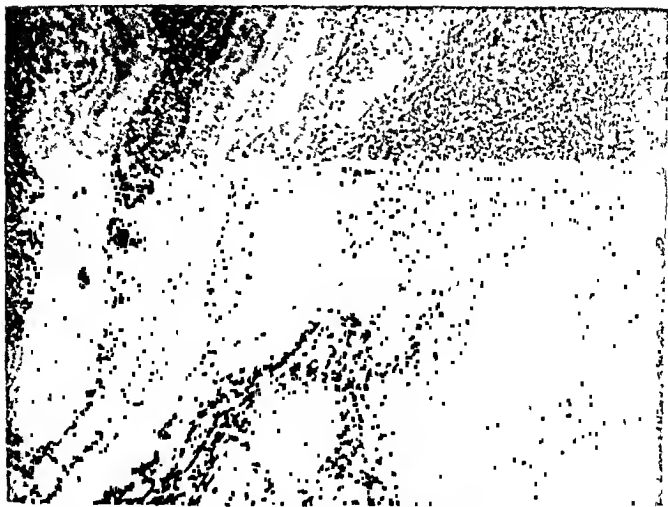
Fig. 1.—Adenolymphoma of parotid gland, showing typical histological structure   ×118
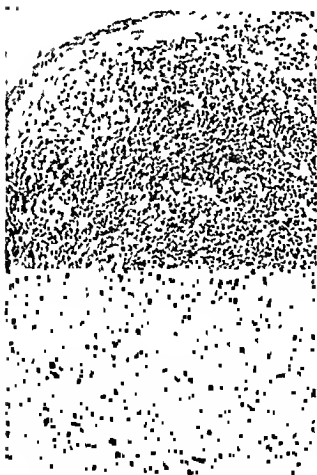
Fig. 2.—Tubercle follicle in the lymphoid constituent of the tumour.   ×118
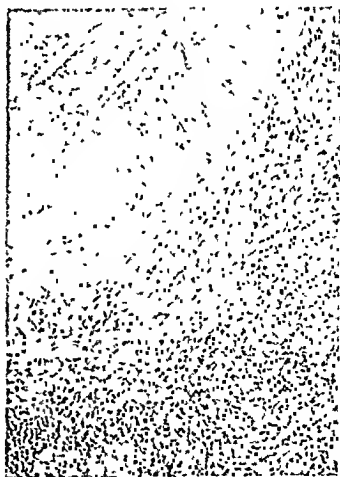
Fig. 3.—Obsolescing caseous tuberculous lesion in stroma of tumour. Cholesterol clefts in caseous area.   ×118

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AN INTRAMURAL CARDIAC ANEURYSM

N H MARTIN

From a Military Laboratory

(PLATE XLII)

The following is an account of a rare, if not unique, cardiac lesion found at autopsy on an apparently healthy soldier who collapsed at work and died within an hour

History

T L, a dispatch rider aged 20 years, had been known personally to his platoon commander and unit medical officer for 18 months They had always considered him absolutely fit Ho had served with his unit throughout the campaign and had never boon off duty from either injury or ill health On the afternoon of his death he had been doing routine duties about the camp and at 4 45 p m he was found unconscious by the roadside close to the camp, his face congested and blue and his breathing stertorous No one had seen him fall Ho was carried to his billet by his comrades, where ho recovered slightly, shouting and struggling violently Ho died at 5 p m while being removed to hospital

Post mortem examination

This was carried out at 11 p m on the day of death

External appearances The body was that of a well built young adult The head and neck were cyanosed There were no external marks of violence

Internal appearances Except for intense generalised congestion, particularly in the lungs and liver, and engorgement of veins, no abnormality was found in any organ except the heart

The *pericardium* was distended with about 20 oz of dark fluid blood There was a circumscribed ring of fibrin on the visceral pericardium around a small, pale swelling on the lateral wall of the left ventricle otherwise inflammatory changes were absent

The *heart* weighed 310 g Both ventricles were contracted The heart was opened along the left lateral border by a cut passing vertically through this swelling, which was found to be the pericardial wall of a cavity 25 mm in diameter lying in the substance of the ventricular myocardium 27 mm from the atrio ventricular ring (fig) The wall of the sac where it bulged out from the ventricle consisted of the pericardium, a thin layer of fibrous tissue in which no muscle fibres could be identified, and the endothelium lining the cavity A small ragged tear in this fibrous wall connected the sac with the pericardial

cavity. The superior, inferior and endocardial walls were bounded by myo-cardium. When first opened, the sac was filled with fluid blood and when this had been gently washed away a few threads of ante-mortem clot were noticed, firmly adhering to the walls of the sac. On exploring the endocardial wall of the sac, a small valve-like opening was found communicating with the cavity of the ventricle. A continuous smooth endothelial lining extended from the ventricle through the opening into the aneurysmal sac, where it had been thrown into folds by contraction of the muscle fibres round it. Beneath the lining, small areas of hæmorrhage were seen in the layer of loose fibrous tissue which separated the lining of the sac from the myocardium.

All the valves were normal and there was no evidence of old endocarditis. The myocardium of the left ventricle was firm and healthy and there was no evidence of hypertrophy, the wall measuring 15 mm. in thickness at the mid-point between the atrio-ventricular ring and the ventricular apex. There was no abnormality in any other part of the heart or in the great vessels. The coronary vessels were normal in structure and distribution. The anterior descending branch of the left coronary artery ran down in front of the aneurysmal sac but had no connection with it, nor was any other connection demonstrable.

Histology

Blocks of tissue were taken from the wall of the sac and adjacent heart muscle, including the wall of the communicating channel between ventricle and sac, from the tissue lying between the anterior descending branch of the left coronary artery and the sac and from areas of apparently healthy ventricular wall some distance from the sac. They were stained with hæmatoxylin and eosin, hæmatoxylin and van Gieson, and Masson's trichrome. The sac was lined by a single layer of flattened endothelial cells, directly beneath which lay loose fibrous tissue separating it from the normal muscle fibres. In one area an organised thrombus could be seen and there was disruption of the lining. The communicating channel was also lined by flattened endothelium, but without interposition of fibrous tissue between it and the heart muscle. In the tissue lying between the descending branch of the left coronary artery and the sac a small patent arteriolar twig was identified. The rest of the ventricular muscle and the small coronary vessels were normal.

Discussion

The immediate cause of death was cardiac tamponade by the hæmo-pericardium resulting from rupture of the thin fibrous pericardial wall of the aneurysmal sac. This was so thin that the patient must have been for some time in imminent danger of this catastrophe. The degree of fibrosis in the wall suggested that the malformation was one of some standing, but there is no reason to suppose that the sac in this anatomical position should produce symptoms, as it was a strictly localised lesion, while the rest of the cardiac muscle was healthy and the blood supply normal. The only way in which the diagnosis might have been established would have been by careful screening.

Intramural aneurysm may result from (1) herniation of the endocardium due to a developmental myocardial defect; (2) endocardial herniation into a localised area of myocardial fibrosis resulting from antenatal trauma, infection or localised coronary occlusion; (3) true coronary aneurysm such as has been described by Cox and Christie (1930); (4) solitary abscess. There was no evidence in this case to suggest that the lesion could have resulted from causes (3) or (4).

Vaquez (1921) mentions, without details, one example of cardiac aneurysm resulting from distension of a localised area of myocardial fibrosis, the legacy of

PLATE XLII

Developmental aneurysm of the left ventricle, showing the smooth lining of the communication between the aneurysmal cavity and the ventricle. The thin fibrous pericardial wall of the cyst and a small fragment of ante mortem clot still adhering to its floor are also seen

old trauma Joachim and Mays (1926-27) describe a second case with a similar background In each instance the aneurysm was sited anteriorly, more or less directly under the chest wall, and the connection with the ventricular cavity was by a relatively large aperture. In both, there was a very clear cut history of substantial trauma. In the present case the sac was sited on the left lateral surface of the heart in a position where it is difficult to imagine the production of localised cardiac damage without associated trauma to other viscera. Of this there was no evidence, nor was there any history or evidence of previous injury or ill health.

Coronary thrombosis, the usual cause of cardiac aneurysm in later life, is excessively rare in a man of 20 (White (1944) says he has seen no case under 22 years of age) and there is no evidence to support a hypothesis of coronary disease or of trauma There was no evidence elsewhere in the heart of acquired endocardial or myocardial disease. Though Abbott (1936) in her classical work makes no reference to a congenital abnormality of this kind one is forced to the conclusion that this sac was in fact the result of a developmental myocardial defect with endocardial herniation.

Summary

An intramural aneurysm of the heart, presumably developmental in origin, is recorded, which was responsible by its rupture for the immediate death of an apparently healthy young adult.

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OBITUARY NOTICES OF DECEASED MEMBERS

Cecil Price-Jones
1863-1943

(PLATE XLIII)

CECIL PRICE-JONES died at his home at Radlett on the 27th of August, 1943, in his 80th year. He was the son of a gifted physician who practised at Surbiton and who, in his earlier years, had held the post of demonstrator of zoology at University College under Ray Lankester. So it was that Cecil did his preliminary training in the pre-clinical years at University College and then in 1880 he entered Guy's for the clinical period. He was recognised as an able and industrious student with an original turn of mind, but in a school that could find no scope within its walls for the genius of Hopkins or Starling there was no opportunity for those whose interests lay outside the purely clinical field. After qualification he studied for some time in Frankfurt and then returned home to join his father in practice. General practice however did not appeal to him and when his father died he left it and devoted himself to pathology, while also acting for a period as medical officer of health for Kingston. There were then few laboratories where bacteriology was taught; each man picked up what he could for himself, and Price-Jones worked in various places. In 1900 he was in the pathological laboratory at King's College and in that year published his first paper, "Cultures of streptotrichiæ from various sources" in the *Transactions of the Pathological Society of London*. Two years later he was with A. G. R. Foulerton in the bacteriological department of the Middlesex Hospital and with him published another paper, on the "general characteristics and pathogenic action of the genus *Streptothrix*". In 1904 he settled down with Eyre, who had just been appointed lecturer in bacteriology at Guy's Hospital on the death of Washbourn. Here he worked as unpaid demonstrator in bacteriology, but also turned once more to the development of the blood, a subject which had engaged his interest in his early days in Frankfurt and now received additional stimulus from the advent of Romanowsky stains. His first paper on the development of the blood cells in the chick was held up by an attack of Malta fever which he contracted when carrying out some cerebral inoculations of mice. This resulted in a long illness which kept him away from work for more than a year. When he came back, he joined Boycott who had been appointed lecturer in experimental

pathology at Guy's Hospital and was then working on the general pathology of the blood. Boycott was investigating the oxygen capacity and the blood volume in various forms of anæmia and Price-Jones studied the changes in the morphology of the cells, noting the difference between the anæmias due to hæmolysis and those due to hæmorrhage. This had led him to the examination of the blood in pernicious anæmia and to a realisation of the need for an accurate method of measuring the size of the red cells. His original apparatus for this was somewhat primitive. He worked on one side of a kitchen table in a room that was half passage between the post-mortem room and Boycott's laboratory; the shelves and benches were full of pots of post-mortem material and underneath the benches were large tall jars containing rats for breeding fleas, with which Boycott was working in connection with an experiment to see how far a flea could jump. Here he arranged a projection apparatus illuminated by a carbon arc lamp, and, with his head and shoulders covered with a large photographic cloth which enveloped the whole apparatus, he drew the outlines of the cells projected on to a piece of paper until the light gave out. Then a loud series of expletives announced that the carbons wanted readjusting and Goodhart, who was engaged with histological matters on the other side of the table, had to down tools and readjust the carbons. As this happened about every ten minutes the method was extremely tedious; it was also extremely warm and P.J. emerged from his wrappings in a state of exhaustion.

When Boycott left Guy's for Manchester, P.J. decided that it was time he earned a little money and he took a post in the Cancer Research Laboratories at the Middlesex Hospital, but his work here was soon to be interrupted by the first German War and in 1915 he joined the R.A.M.C. and went to France to take charge of the laboratory at no. 14 General Hospital at Wimereux. Here he was a great success and he greatly surprised his clinical colleagues by the help he was able to give by means of the blood picture. He also took great trouble in arranging entertainments for the troops and especially concerts to which he himself made notable contributions with his 'cello.

By the end of the war Boycott had returned to London, to the chair at University College Hospital Medical School. Here Price-Jones once more joined him and, under his stimulating influence, continued his studies of the size of the red blood cells. Here he was able to work under much more favourable conditions and by his patient and time-consuming studies demonstrated not only the importance of measuring the size of the red cells in distinguishing different varieties of anæmia but also the value of applying accurate statistical methods in the problems of pathology.

He thus made clear the fact that pernicious anæmia was a megalocytic anæmia some years before Minot and Murphy's first paper on the curative effect of liver treatment, and he indicated that pernicious anæmia was due to faulty blood production and not to

excessive blood destruction, the view that was then almost universally accepted as a result of William Hunter's work.

He also did a great deal of work in establishing the normal figures for the red cell and hæmoglobin content of the blood in man. It had long been recognised that the figures for hæmoglobin found by American workers were higher than those in England as measured by the Haldane method. To his direct mind there was only one thing to do. He packed his bag and went to America and at Boston, where Payling Wright was then working, and with the latter's help, he arranged to investigate a group of healthy individuals of the same standing as those on which the English figures were based. In this way he established the fact that the red cell and hæmoglobin values for the Boston group were higher than the London ones.

P.J. was a man of wide culture and had many interests outside pathology. He was a great traveller and lover of the sea. Occasionally he used the great liners, but he preferred the more leisurely journey on a cargo ship, where he could pass the day reading and sketching, and the many lovely pencil and water-colour sketches that adorned his walls were a constant reminder of his many travels. At one time he had a bungalow at Walmer and sailed his own boat; here too he could indulge his love of flowers and he planted and cultivated his own garden. In later life he turned to this again, building himself a house at Radlett on the summit of an apple orchard where in the spring he looked down on the orchard beneath, where the apple trees with a liberal sprinkling of cherry and plum provided a fairy-like picture. He was no mean musician. In boyhood he was devoted to the piano but one day his father came in when he was practising and slammed down the lid of the keyboard and forbade him to touch it again. When the time came that he could take it up once more he felt that his hands lacked the suppleness to make him a good player, so he took up the 'cello, and, although he played the piano with great pleasure to his friends, his "Strad" remained his most cherished possession. P.J. although a man of spare habit appreciated good food and had a remarkable palate for wine. This was once well demonstrated when he was in practice with his father. His father had a bosom friend who was also his wine merchant and a frequent guest at dinner. On one occasion after the purchase of some port P.J. said that the wine was not the same as the sample bottle; so when the wine merchant next came to dinner the father said that though he himself could not detect the difference Cecil said that it was not the same as the sample. The wine merchant was furious and denied it. P.J. said there was still some of the sample left and he would be glad to prove it. A dozen glasses were brought in and when they had been charged P.J. returned to the room and successfully picked out the sample glasses. The story has a sad ending, as the wine merchant took it badly and the friendship ended abruptly.

In his later years he suffered a good deal from attacks of dyspnœa

and " cardiac " pain but continued working until one day he slipped when walking up from his lily pond to the house and fractured his femur. This resulted in several months in the private wing at U.C.H. Hardly had he got back home when he had an attack of biliary colic. He immediately returned to U.C.H., where the surgeon relieved him not only of his gall stones but also of his " cardiac " pain! But for the rest of his life he could only walk with difficulty and was then cut off from most of his old friends. Sir Edward Salisbury, whose frequent visits at this period were a great joy to him, writes :—

" P.J. possessed a highly educated taste both in the metaphorical and the literal sense of the phrase. Whether it were the use of a word, the balance of a musical composition, the tone value of a picture, the flavour of an apple or the bouquet of a choice Hoek, he gave them alike considered attention and that combination of æsthetic and intellectual appreciation that is the hall-mark of the true connoissseur.

" The enforced isolation of his later years was felt not only because it cut him off from so many of his friends, especially during the war, but because it deprived him of new scenes and experiences. He was rarely averse to talking of his travels and the sketches which he had made on them. His pencil sketches especially showed a lively sense of atmosphere and he was more successful in this medium than in water-colour. The charm of his playing whether on the piano or the 'cello was not to be measured by his technical ability, though this was appreciable. To hear P.J. extemporise for an hour or more on the keyboard was to appreciate the delicacy of his perceptions and share the pleasures of an artistic mind. Though P.J.'s scientific achievements will endure the longer, it is the artistic side of his temperament that will remain very vivid to those who knew him personally.

" He was keenly interested in the way the world was made and often questioned me on botanical and other scientific subjects. But one felt that though the warp and woof of life had for P.J. its attractions, it was its texture and the play of light and shade upon it that was the absorbing interest ".

G. R. Cameron writes :—" P.J., as he was called with some affection by all University College Hospital Medical School colleagues, occupied a unique position in the School. Though not on the staff, he was regarded as the final court of appeal on hæmatology and his opinion was sought by both clinicians and medical students. No one thought of commencing a research on blood without first consulting him. Visits had a habit of being prolonged into a lengthy gossip and his laboratory at times resembled a club room. The little man, perched on one of the enormously tall stools (Boycott had planned laboratory furniture to suit his own very long legs), was the focus of many a witty conversation. P.J. got on well with young people despite the wide discrepancy in age. He could talk to them in their own language, yet he never seemed to be " talking down ". Though

ever friendly and mildly encouraging, he could on occasion be roused to indignation, but his stutter inevitably intervened and threatened to turn the flow of biting invective one felt was coming into a comic situation. The stutter on one occasion saved what might almost have turned into an international situation. We were visiting Rome for a Congress on the History of Medicine and amongst the various social gatherings was a reception at the Vatican. With due ceremony we were lined up in a double row to await the advent of the Holy Father. Preceded by the Cardinal on duty he made the circuit of the visibly impressed visitors, each of whom was introduced in turn. P.J. was solemnly announced as 'Prince Jones', much to his dismay and the amusement of at least one witness. He attempted to correct the vicarious ennoblement, but the stutter intervened and no doubt the papal archives record to this day the presentation at the Papal See of a Royal visitor from the Principality of Wales.

"That visit to Rome must have been a great joy to him, for he revelled in sketching and spent hours in the Forum and at Tivoli with his pencil and sketching book. A memorable outing to Monte Cassino brought its reward in glorious Italian scenery and a striking sunset; it was not without its ludicrous moments, however. Primitive sanitary arrangements in Italian hill towns, the enormous mounds of spaghetti which had to be dealt with, the incessant chatter of our French colleagues and their wives, not to mention the ridiculous poodles which invariably accompanied them, presented their problems and P.J.'s eyebrows rose higher and higher until they threatened to disappear completely. And to crown all was the presentation after lunch to the Abbot of Monte Cassino. The faithful solemnly kissed the episcopal ring which decorated the podgy fingers of that high dignitary. P.J. manfully summoned up his courage and grasped the slightly bewildered Prince of the Church by the hand. Those of us who followed, faced with the same problem, breathed a sigh of relief and overwhelmed the Abbot with a typical British gesture ".

Dr Janet Vaughan writes :—" The name of Price-Jones will endure in medicine attached to a curve—namely the distribution curve of red cell size. It is indeed fitting that one of the first men to recognise how much might be gained by the application of precise statistical methods to the biological sciences should have insured himself such immortality. By plotting the distribution curve of red cell size in different pathological conditions Price-Jones was able to show that the curve found in Addisonian pernicious anæmia was distinctive and almost diagnostic. He made in fact of statistical measurement an essential clinical tool, needed at the bedside as well as in the research laboratory.

"His first paper on the subject, published in 1910, was followed by a succession of further papers in this *Journal* dealing not only with cell sizes but with other hæmatological constants. In 1933 he summarised his observations in a classical monograph entitled 'Red

blood cell diameters '. He had shown in the intervening years not only that measurement of cell size was of diagnostic value but that it was also a valuable guide to the effects of treatment.

" Price-Jones will be remembered for his curve, but the real importance of his contribution to medicine lay in the fact that he taught to his own and succeeding generations the importance of both accurate measurement and the correct assessment of the significance of results. He taught us that we cannot claim anything to be abnormal unless we know what are the limits of normal variation. What he did for red cell size and hæmoglobin is gradually but all too slowly being done for other fields of pathological enquiry ".

But Price-Jones was no mere " hæmatologist ", as some who only knew him in his later years have thought; he was a pathologist in the true sense of the word, with wide experience buttressed by wide reading. A clear thinker, with a keenly critical sense and a precise and scholarly pen, his abstracts and reviews, of which he wrote many for various journals, were always full of wise comment and were a delight to read. He was an assistant editor of this *Journal* from 1923 to 1926 and had been a member of our Society since 1908.

<div align="right">G. W. GOODHART</div>

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Elizabeth O'Flynn (Lady Stanton)

Dr Elizabeth O'Flynn died from influenzal bronchopneumonia in the Hospital of St John and St Elizabeth on 1st February 1946. During the last few years of her life she had been much handicapped by ill-health but she struggled on against great difficulties and was actually working until within a week of her death. For more than twenty years she had held the post of pathologist to the Queen's Hospital for Children, and before this had held pathological appointments at the National Hospital, Queen Square, the South London Hospital and King's College Hospital. These had given her special experience in the pathology of diseases of the nervous system and

in collaboration with her colleagues she published papers on subacute combined degeneration, the calcium content of cerebrospinal fluid and the hepatic aspect of lethargic encephalitis. In 1930 she married Sir Thomas Stanton, K.C.M.G., Chief Medical Adviser to the Secretary of State for the Colonies, but her husband died in 1936, and it seemed to her friends that she never recovered from the shock of his death.

Her loss will be felt by a large circle of friends, because she had a great capacity for friendship. She was always inclined to belittle her own achievements in pathology, but those who knew her well had a great respect for her opinion on a difficult problem. She often found the obligations of routine duties a strain, because she was inclined to be rather casual and happy-go-lucky by nature, but she continued in her hospital work with very little technical assistance, inspired by a strong sense of duty.

CUTHBERT DUKES

BOOKS RECEIVED

Biological actions of sex hormones

By HAROLD BURROWS. 1945. Cambridge: At the University Press. Pp. x and 514; 7 text figs. 42s.

No one can know better than the author how essential is a knowledge of the biological actions of sex hormones to both experimentalist and clinician. He modestly says in the preface that " our knowledge of these matters is growing so fast that to keep abreast of it is not easy for those who are occupied with many other affairs ". This statement might well be modified by saying that it is quite impossible even for a research worker to sift the important from the unimportant in this rapidly expanding field. It is to such a book as this, covering nearly 2000 references during a period of 20 years, that we look for inspiration and guidance. The author approaches his task with a background of laboratory experience and philosophical thought which fit him peculiarly for the task in hand. System, accuracy and limitless patience have contributed to produce a complete factual account of the experimental work which has been done. It is therefore unfortunate that the author has so seldom thought fit to express his own views upon the value of the work quoted.

This is not a book to read from cover to cover, enlivened as it is by an occasional gleam of humour; rather is it a work of reference to be kept at hand in the laboratory or consulting room. It is divided into six parts, each dealing with a group of hormones :—gonadotrophins, gonadal hormones (androgens, œstrogens and progestins) and finally sex hormones of the adrenal cortex. The experimental work leading to the discovery and isolation of the hormones is described, followed by a survey of all their complicated biological actions and interactions. The relations of hormones to pathological conditions such as non-descent of the testicle, hernia and mammary cancer are also discussed. An appended list of proprietary sex hormone preparations and synonyms is a useful addition.

The format conforms to the very highest war-time standards. There are many tables but no illustrations. Any attempt to include the latter, even if helpful to the reader, would have been prohibited by present paper restrictions.

Pulmonary edema and inflammation

By C. K. DRINKER. 1945. Cambridge, Mass; Harvard University Press : London; Humphrey Milford (Oxford University Press). Pp. ix and 106 ; 27 figs. $2.50.

This small book is based on the Nathalie Gray Bernard Lectures delivered by Dr Drinker at the Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, North Carolina, in December 1944. A chapter has been added on artificial respiration. The main theme of the book is the part played in the causation of pulmonary œdema by changes in the permeability of the capillary endothelium of the lung and especially the role of anoxia in effecting these changes.

The capillary circulation of the lungs is a close-meshed net embracing the alveoli so intimately that they appear to be suspended in an almost continuous cascade of blood. Such a vast capillary area suffices for the

extremes provided by the heaviest physical exercise; it can also be cut down to the slightest proportions when respiratory needs are no longer urgent. The capillary endothelium depends for its oxygen supply on the air which reaches the alveoli, hence anything which excludes air from groups of alveoli leads to anoxia and this in turn gives increased capillary permeability with outpouring of plasma into the air spaces. Although an extensive lymphatic system exists in the lungs it is confined almost wholly to the bronchial ramifications, ceasing abruptly at the infundibular ducts; moreover, most of the lymphatics drain into the right subclavian vein by way of the right lymphatic duct, which is very small and constitutes "a veritable bottleneck" in the pathway of lymph drainage. Quantitative studies such as those of Dr Drinker's school and unpublished experiments of F. C. Courtice in this country do not impress one with the functional efficiency of the lymphatic system in the lungs. Stagnation of exudates or transudates is thus favoured, anoxia is again likely and a vicious circle may well result. Leakage of water from the lung capillaries is favoured, too, by the negative pressure in the chest, and if this be increased as by dyspnœa œdema may be precipitated.

Two lines of therapeutic attack upon pulmonary œdema emerge from these principles: (1) the combating of the progressive effects of anoxia; (2) the modifying of intrapulmonary pressure conditions so that negative thoracic pressure is reduced.

Dr Drinker is to be congratulated upon a singularly clear discussion of a difficult subject. His lectures should be in the hands of all teachers and research workers interested in pulmonary problems.

The role of the leucocytes of the blood in reparative processes in the tissues

By G. K. Kruschov. 1945. Moscow: Publications of the Academy of the Sciences of the U.S.S.R. Pp. 116: 20 text figs. 5 roubles. [In Russian.]

Professor Kruschov, in the Russian Institute of Cytology, Histology and Embryology, has repeated, continued and elaborated Carrel's experiments on the influence of the leucocytes upon tissue growth in culture and upon healing wounds in the living mammal, and has arrived at certain conclusions regarding the mode of action and attributes of the "leucocytic trephones" —those leucocytic products which appear to stimulate regeneration in connective and epithelial tissues.

Several methods were employed for the collection of the leucocytes: (1) centrifugalisation (a film of leucocytes was obtained from the interspace between plasma and cells); (2) slow gravitation (a suspension of white cells in plasma was collected after sedimentation of the erythrocytes, and this suspension was centrifuged to provide a deposit of white cells); (3) perfusion of the spleen. To separate polymorphonuclear from lymphocytic forms, advantage was taken of the more rapid motion of the former, through a plasma medium, to pieces of elder pith. Kruschov shows that the growth of a tissue culture (fibroblasts) or an organ culture (cornea) is accelerated by trephone-containing fluid almost but not quite to the same degree as by embryonic extract. The transplantation of leucocytes themselves to a tissue culture stimulates the rate of growth substantially more than the addition of trephone-containing fluid and rather more than the addition of embryonic extract. Perhaps the clearest demonstration of the stimulant effect of leucocytes is afforded by an experiment in which a transplanted colony of leucocytes is placed co-tangentially with a tissue culture; growth of the culture at the area of contact is visibly more rapid

than the growth of a control culture separated from the leucocyte colony by a distance of a few centimetres

The accelerating effect of leucocytes and of leucocytic trephones was observed also in the regeneration of an area ablated from a tissue culture, and in addition it was shown in the repair of traumatic ulcers on the back of rats, carefully controlled by identical contralateral ulcers untreated by leucocytes or trephones In the healing of these ulcers the leucocytes exert an accelerating effect both on early granulation production and on later epithehalisation The differences in rate of growth and in regeneration are graphically represented and are apparently statistically significant The action of leucocytes and their products was found to be in no way specific—for the tissue, the protein content of the medium employed, or the species

The final chapters of the book report the early application of these stimulating attributes of leucocytes and their products to veterinary and human medicine, but controls have been so difficult to obtain that final conclusions particularly in the case of man, cannot as yet be drawn from the clinical experiments

Micro-analysis in medical biochemistry

By E J KING 1946 London J & A Churchill, Ltd Pp viii and 168, 16 figs 10s 6d

Hospital biochemists will welcome this collection of laboratory methods For the most part the methods are those with the development of which the author and his colleagues have been closely associated and which have already gained a wide measure of acceptance, and it is a distinct convenience to find them gathered together in one small volume The author's intention has been to supply instructions which will make it possible to execute all the investigations commonly asked for in routine hospital laboratory work In fulfilling this intention he has confined himself in the main to one method for each determination Thus the book, while being a most useful laboratory companion, is not a complete laboratory guide

Some notes on the clinical interpretation of results are given, but these are very brief In the reviewer's opinion, unless clinical interpretation is discussed at some length it is best omitted altogether Short notes such as are found here are quite likely to mislead any beginner who may seek guidance from them, and they are valueless to the experienced worker

Fungicides and their action

By JAMES G HORSFALL 1945 Waltham, Mass , The Chronica Botanica Co London William Dawson & Sons Ltd Pp xvi and 239 , 24 text figs $5

This book by Professor Horsfall, Head of the Department of Plant Pathology and Botany at the Connecticut Agricultural Experiment Station, deals in a successful and comprehensive way with the scientific principles underlying the chemical control of fungus diseases of plants and includes references to work in other spheres such as the preservation of timbers and of fabrics Accounts are given of the many types of materials now in use as fungicides or under investigation but the book is in no way a compendium of chemical formulations It is essentially a book for the specialised scientist and will have little appeal to the general grower

During recent years the position with regard to materials used for plant disease control has been rapidly changing New types of compounds of fungicidal value particularly of an organic nature, have been found and

some of these are already challenging the older and well established inorganic remedies. In making these advances much work has been done and Professor Horsfall's book serves a useful purpose in drawing this together and in permitting an assessment of the present position.

The book forms the second of the series in the Annals of Cryptogamy and Phytopathology edited by Dr Frans Verdoorn. Chapters are devoted to an historical introduction, general concepts, laboratory assay, problems of data assessment, principles of chemical protection, deposition, the coverage of surfaces, tenacity, artificial immunisation and chemotherapy, antagonism and synergism, phytotoxicity and the action of copper, sulphur, organic nitrogen and other organic compounds. Technically the book fully maintains the high standard set by the first volume in the series, while the lay-out is attractive and the type is conducive to easy reading. It contains a short foreword by Dr David Fairchild, author and subject indexes and a bibliography of some 500 references.

The book will prove a most useful addition to the libraries of all who are interested in plant pathology. The chapters on data assessment, the new organic fungicides, and antagonism and synergism are of particular value at this time in view of the recent interest in these matters. Some of the contentions put forward by the author are likely to be disputed, but the book will undoubtedly provide a valuable impetus to further work in this important subject. In the reviewer's opinion, the high merit of the book is somewhat marred by the use of colloquial terms and loose chemical phraseology, which, while no doubt making for easier reading, seem out of place in a scientific work of this nature. In some cases, matters dealt with in the text show no references or are inadequately covered in the subject index. Such omissions will doubtless be rectified in a later edition, and should in no way detract from the merit of a stimulating and valuable publication.

The bacteriology, pathology and etiology of measles pneumonia and measles encephalomyelitis with venous thrombosis

By PAUL GATES KREIDER. 1943. Springfield, Illinois : published by the author. Pp. vi and 86 ; 19 figs. and 3 colour plates. No price stated.

In this small monograph the author presents two theses. The first is that the pneumonia of measles is due to streptococci which are, for him, almost a constant concomitant of severe measles infection. It is not due to secondary invasion of damaged tissues but to a concomitant infection, as X-ray evidence shows that bronchitis appears with the fever at the onset of the catarrhal stage. The streptococci causing the pneumonia may be of either the hæmolytic or viridans type, but for the author this is unimportant, as he has found evidence suggesting "that the hæmolytic streptococci had been in many cases partially and in some cases completely converted into the green-forming type". The reader is left with the impression that the author would like to be able to prove that measles is due, not to a virus, but to a streptococcus, but he contents himself with suggesting that further work may show the constant presence of streptococci in the respiratory tract in cases of measles. The alternative hypothesis which would appear to account for the facts brought forward by the author, namely that the increase of mortality as an epidemic develops is due to combined infection by measles and virulent streptococci, is not even suggested.

The second thesis is that the encephalomyelitis of measles is due to venous thrombosis, or at least to a standstill of the venous circulation in the nervous system. This theory is clearly based on Putnam's views

on the causation of disseminated sclerosis, but it does not explain the difference of the histological picture from that seen in extensive primary venous thrombosis in the brain The two cases of measles encephalo myelitis which he describes have already been recorded by Ferraro and Scheffer, and he adds little to what is already known of the pathology of the condition The book is admirably illustrated by drawings and photographs and includes an extensive bibliography.

The sulphonamides in theory and practice

By J. Stewart Lawrence 1946 London H K Lewis Pp vii and 125 9s

This slim volume is intended primarily as a guide to the clinician, and contains a straightforward account of the pharmacology of the sulphonamide drugs and of their therapeutic use Theory ", apart from pharmacology, occupies only the first 11 pages, and recent work on the mode of action of these drugs, showing that the original theory of Fildes and Woods is not a full explanation, is not mentioned The clinical chapters embody some original observations, mainly tending to show that sulphonamide treatment is valueless in certain conditions for which it is commonly prescribed, the author is commendably hostile to indiscriminate use The book ends with a short account of laboratory methods, a list of 324 references, arranged in alphabetical order from " 0 " to 282 only, and a remarkably full index for so small a book

Textbook of neuropathology

By Arthur Weil Second edition, 1946 London William Heinemann (Medical Books) Ltd Pp xiv and 356, 287 text figs 25s

- According to the preface " This book has been designed to give a review of the present state of our knowledge in neuropathology It contains a collection of facts which have been scattered in the literature " This statement gives a clue to the principal merits and defects of the work It does indeed contain many interesting facts, but no serious attempt has been made to integrate them into clear and orderly accounts of the various morbid entities It also contains many novel hypotheses but no sound reasons are given for preferring these to well founded theories of greater antiquity It is further provided with an abundance of illustrations, mostly of excellent quality, and these are its most attractive feature

In the section on the spread of infection into the cranium from neighbouring inflammatory foci the importance of lymphatic pathways is stressed and other paths of extension are almost entirely neglected We are also told that " seldom does a tuberculous meningitis occur together with acute miliary tuberculosis " Which only goes to show that the American way of life is very different from the European—even for the pyogenic micrococci and the tubercle bacillus

The allocation of space to the various subjects discussed provokes little comment, but " apoplexy ' is dismissed in two meagre pages while cerebral aneurysm and meningeal hæmorrhage are barely mentioned, although American workers have made substantial contributions to our knowledge of these subjects

The author frequently shows a disrespect for the accepted meaning of terms that is always irritating and sometimes misleading The use of " olfactory bulbs " for " olfactory nerves " (p 101) and of " creatine " for " creatinine " (p 202) can be excused as minor slips, but the substitution of " athoromatous abscess " for " atheromatous ulcer " (pp 81 and 82)

" syndrome " for " sign " or " symptom " (p. 94), " tissue " for " thrombus " (p. 124) and the reference to the " long, fine fibers " of the microglia cell (p. 34) suggest a more reckless glissade towards verbal anarchy.

Nevertheless, in reaching a second edition this book displays a vitality often lacking in similar text-books in our language and we hope that in later editions it may also acquire the equally important virtues of clarity, simplicity and reliability.

Topley and Wilson's principles of bacteriology and immunity

Revised by G. S. WILSON and A. A. MILES. Third edition, 1946. London: Edward Arnold & Co.　Vol. I, pp. xi and 970, 235 text figs ; vol II, pp. viii and 1084, 67 text figs.　60s.

The impatience with which a new edition of " Topley and Wilson " has been awaited by bacteriologists is some measure of the success achieved by the two earlier editions.　G. S. Wilson and A. A. Miles (who has under-taken the revision of the sections for which Topley was mainly responsible) are to be congratulated upon having brought the book thoroughly up to date without impairment of the balanced presentation of the original and in spite of the difficulties occasioned by the disorganisation of war.

The incorporation of the notable advances of the last decade has resulted in a considerable increase in the size of the book but reversion to a two volume format makes this edition much easier to handle than its too bulky predecessor, whilst cross reference is facilitated by the inclusion of a complete index to the whole work in each of the volumes.　Two entirely new chapters, on chemotherapy and the bacteriology of the air, have been added.　The former, which includes a critical discussion of current hypo-theses of the mechanism of antibiotic activity, provides a most valuable review of the present-day position of its subject ; the latter deals mainly with modern methods of preventing air-borne infection in closed spaces. One useful chapter of the earlier editions—on soil bacteriology—has unfortunately been deleted without the substitution of anything to take its place in giving the student an appreciation of the significance of bacteria in the economy of nature.　The Shigella and Salmonella organisms have been removed from the Bacterium chapter and are now treated separately as distinct genera.　Also the psittacosis-lymphogranuloma group has been separated from the other virus diseases.　Several new figures have been added, including some beautiful electron photo-micrographs to illustrate the morphology of bacteria, spirochætes and viruses.　Apart from these and a few other minor changes the form of the book remains unaltered.

The critical appraisement of very recent work and its correlation with established hypotheses is always a difficult task and there is little doubt that future editions will benefit from vigorous pruning.　The revisers themselves are conscious of this, for they plead in the preface that they have not had time to be more concise.　There are some sections, notably that dealing with the influence of diet etc. on immunity, in which detailed discussion of directly contradictory reports tends to obscure rather than clarify the issues involved.　The chapters dealing with viruses and virus diseases fall somewhat short of the high standard of the rest of the book. The advisability of discussing the general properties of animal viruses with scarcely any reference to the plant pathogens is open to question.　The use of the developing hen's egg in virus work is not given the attention which its importance merits ;　the allantoic route of inoculation, so successfully employed for the large-scale production of some virus vaccines, is not even mentioned in the paragraphs devoted to virus culture.　Over

30 virus diseases have been added to the formidable number dealt with in the previous edition but the evidence for a virus aetiology of some of them, such as Bornholm disease and epizootic abortion of mares, is so slender that they might with advantage have been excluded Of course in a work of such wide scope it is inevitable that specialists in limited fields will find something with which to disagree None the less, in its new form, this book will undoubtedly continue to serve, both as a text book indispensable to advanced students and as a reference book equally indispensable to teachers and research workers The long lists of references appended to each chapter offer to the investigator in almost any branch of bacteriological research a ready means of entry into the literature of his subject

Penicillin therapy and control in 21 Army Group

1945 Published under the direction of the Director of Medical Services, 21 Army Group, with introduction by the Consulting Surgeon (Brigadier A E Porritt) Pp 365, 4 figs No price stated

This publication of 365 pages is made up of some 60 contributions or short chapters by a great number of medical men, working as pathologists, surgeons and physicians in the 21st Army Group The objective to which most of these articles are directed is the assessment of the value of penicillin in the prophylaxis and treatment of war wound infections, but the collection also includes valuable contributions to our knowledge of the use of this drug in infections treated in the medical wards of Army hospitals, and, further, there are accounts of several useful investigations on the technique of penicillin administration The latter cover many aspects of the use of this drug, from observations on the destruction of penicillin by various samples and varieties of rubber tubing with which it is likely to be in contact in drip administration, to the concentrations of the drug which may be obtained in the blood, the synovial fluid of joints, etc , of patients under treatment These contributions to the technical side of the subject come mostly from Major K E A Hughes of No 3 Mobile Laboratory

The individual records from different hospital units bearing on the outcome of prophylactic treatment of wound infections vary considerably in scope and value, some being too limited to permit of definite conclusions, but the sum total presents a most convincing proof of the remarkable advance, even during the course of the War, in the protection of the wounded from the grave results of infection It is shown that even when just allowance is made for other factors which bear on this result there can be little doubt that the parenteral administration of penicillin for purposes of prophylaxis has yielded a rich harvest in lives saved and in the elimination of protracted illness and disablement It is also made clear that results with penicillin alone are as good as those obtained by a combination of penicillin and sulphonamides and definitely superior to those obtained with sulphonamides alone

One of the most interesting chapters is that by Brigadier A E Porritt and Lt Col G A G Mitchell on anaerobic myositis The incidence of this condition in the wounded, which had ranged from 3 4 to 10 per 1000 in the campaigns of the Middle East, N Africa and Italy, was reduced in the B L A to 1 6 per 1000 in Allied troops , and the death rate in established cases, which fluctuated between 30 and 70 per cent in other areas in earlier campaigns, fell to 21 9 per cent The figures are not, however, large enough to throw much light on the outstanding problem of the value of prophylactic anti serum for gas gangrene, especially as both this and penicillin were given to so many of the wounded

A failure is recorded by Lt.-Col. R. E. Tunbridge and Majors J. H. H. Keall and J. V. Dacie, in an attempt to accelerate the clearing of diphtheria carriers by the local administration of penicillin as spray and lozenges.

The whole presents an account of medical scientific work of a high order carried out often in conditions of peculiar difficulty and yielding many valuable results.

Virus as organism : evolutionary and ecological aspects of some human virus diseases

By FRANK M. BURNET. 1945. Cambridge, Mass. ; Harvard University Press : London ; Humphrey Milford (Oxford University Press). Pp. vii and 134. $2.

This small volume is an expansion of the Dunham lectures given at Harvard University in 1944. As the sub-title indicates, the lectures are a discussion on human virus disease from the point of view of an ecological interaction between virus and man. In the earlier chapters the author discusses, in a general way, reproduction, variation and survival of viruses, evoluton and change in virus disease and the reaction of the host to virus infection. He accepts the view put forward by Laidlaw and Green that viruses are micro-organisms which have evolved by parasitic degeneration from larger micro-organisms, many of them in all probability from bacteria. Viruses reproduce by replicating their structure at the expense of suitable atomic groups within the living substance of susceptible cells and the capacity of viruses to vary is discussed in relation to the adaptation of viruses to new hosts. From what is known of the evolution of virus diseases it is suggested that the selective survival of new mutants may within a short period produce a new dominant type of disease. The author points out that transfer of virus from animal reservoirs to man is taking place at present and puts forward strong arguments in support of the view that in the past nearly all virus diseases of man must have been derived from animal infections. The author's views are illustrated in separate chapters on herpes simplex, poliomyelitis, psittacosis and related infections, smallpox, alastrim and vaccinia, yellow fever and influenza. This is a fascinating little book written by an authority whose observations always merit serious consideration.

Microbiology and epidemiology

Edited by E. B. BABSKY, I. G. KOCHERGIN and V. V. PARIN. Translated from the Russian by H. P. Fox. 1945. London : Medical Publications Ltd. Pp. 158. 15s.

This little book, first published in the U.S.S.R. in 1943, gives in fifteen chapters by various authors an account of work carried out in the Soviet Union on the prevention and treatment of typhus, cholera, tularæmia, the enteric fevers, dysentery, tetanus, gas gangrene and certain virus infections of the central nervous system. Special attention appears to have been focussed on the preparation of bacteriophages for use in prophylaxis and treatment, but with regard to the infections concerned, no convincing evidence is produced as to the efficacy of these phages in practice. Chapters are devoted to the control of pediculosis, to gramicidin and to penicillin and a short account of antibiotics from plants (phytomicides) is included. In some sections, such as those on typhus and tetanus, the work of Soviet laboratories has been discussed in relation to advances made in other countries ; in other chapters only the results achieved by Soviet investigators are considered.

PROCEEDINGS OF THE PATHOLOGICAL SOCIETY OF GREAT BRITAIN AND IRELAND

29th and 30th March 1946

The seventy first meeting of the Society was held in the University of Sheffield on Friday and Saturday, 29th and 30th March 1946

Communications and demonstrations

These marked with an asterisk are abstracted below

R. E. O. WILLIAMS and G. J. HARPER. Staphylococcal hæmolysins on sheep-blood agar plates.

J. UNGAR. The effect of penicillin on the growth of the human type of *M. tuberculosis.*

*A. E. FRANCIS. Two new types of *Shigella flexneri.*

C. L. OAKLEY, G. HARRIET WARRACK and PATRICIA H. CLARKE. The toxins of *Clostridium œdematiens.*

*H. McILWAIN The metabolism of glutamine by micro organisms in relation to the need by hæmolytic streptococci for added glutamine in growth.

H. N. GREEN. The effect of environmental temperature on ischæmic and ndenosine triphosphate " shock ".

H. B. STONER and H. N. GREEN The effect of burns on the ndenosine equivalent of the blood.

L. C. D. HERMITTE. The diagnosis of tumours by aspiration biopsy.

H. E. HANDINO. Effects in the lungs of kittens given acetyl aminofluorene by mouth

G. R. CAMERON Some effects of acute anhydræmia.

E. J. KING, M. V. RAE and M. GILCHRIST. The effect of sericite, with and without treatment by hydrochloric acid, on the lungs of rats.

T. A. LLOYD DAVIES The pulmonary effects of the inhalation of manganese dioxide.

I. B. SNEDDON. Nutritional neuropathy in prisoners of war.

H. J. BARRIE. Constitutional non-hæmolytic hyper bilirubinæmia.

E. C ARMSTRONG, G. M. BONSER and L. H. STICKLAND. The carcinogenic action of 2-acetyl aminofluorene on various strains of mice.

R J LUDFORD Experimental analysis of the problem of sarcomatous trans formation of the stroma of carcinomata.

L DMOCHOWSKI and E S. HORNING. The action of œstrone on the lymphoid tissues of male mice.

J. W. ORR The induction of carcinoma of the breast in mice with methyl-cholanthrene.

A RENSHAW An investigation into the physical efficiency of coal-miners as deduced from their blood sugar levels.

PATRICIA H CLARKE. The typing of *Clostridium œdematiens.*

G. S. W. DE SANAM and M R V. PIERIS (1) Epitrochlear gland showing an adult female filaria, the uterus of which is stuffed with microfilariæ. *(2) Chorion epitheliomatous tissue discovered in blood clot removed during an opera-tion for supposed inguinal hernia , death six weeks later with widespread generalised chorionepithelioma.

T. Crawford.   An unusual case of polyarteritis and polyphlebitis of the liver.

A. J. McCall.   A case of spontaneous cerebral ventriculostium.

L. C. D. Hermitte.   (1) Pictorial methods in teaching pathology.   (2) Rare material of pathological interest.

H. N. Green and H. E. Harding.   The " pyrophosphato kidney ".

S. L. Baker.   (1) Osteogenesis imperfecta in a child of 17 days.   (2) Islet tumour of pancreas.   (3) Arrhonoblastoma of ovary.

C. H. Andrewes, R. E. Glover and Janet S. F. Niven.   Lesions produced in mice and cotton rats by grey lung virus.

F. Bielschowsky.   (1) Experimental basal cell carcinomata of the skin.   (2) Experimental tumours of the intestine of the rat.

Alan C. Lendrum.   (1) Teratoma of testis, with complex metastases.   (2) Apocrine glands in teratoma of ovary.

Abstracts

<div align="right">

576 . 851 . 49 (*Shigella flexneri*)

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TWO NEW TYPES OF *SHIGELLA FLEXNERI*

A. E. Francis

From the Pathological Department, St Bartholomew's Hospital, London

Type 953 (provisional *Shigella flexneri* type VII)

In June 1944 two cultures of dysentery bacilli isolated from Italian civilians and designated 2·193 and 2·694 were received at the Emergency Vaccine Laboratory, R.A.M.C., from Major W. H. Ewing, Sn. C., U.S. Army, who suggested that they might be strains of Boyd's (1938, 1939-40) type V, P.143. The new strains were found to agglutinate to the homologous titre with the only two P.143 sera available ; but these were of rather low titre, and when another P.143 serum was prepared, it was found that they agglutinated to only one-third of the homologous titre.

Absorption tests showed that absorption of a P.143 serum, having equal titres for both types, with P.143 removed over 80 per cent. of the agglutinin for 2·193, but absorption of the same serum with 2·193 did not affect the titre for P.143.   When sera were prepared with strain 2·193, titres of 2500 and 5000 (trace) were obtained, but these sera failed to agglutinate either of two strains of P.143 at dilutions from 1 : 20, in Dreyer tubes after 4 hours at 50° C. These sera were found to contain agglutinin in high titre for the Flexner group-phase suspensions, Y and 103 B, but much less for 119 B and for X.   The titre of agglutinin for Flexner Y rose in one rabbit from 80 before inoculation to 2500 (trace), and in the other from 320 to 1280.   In two rabbits inoculated with *Shigella sonnei*, phase II, no such rise of the " normal " Flexner group-phase agglutinin took place.   Further, when one of the sera was absorbed with either 2·193 or 2·694, this Flexner group-phase agglutinin was removed as well as the specific agglutinin, and it was later shown that strain 953 (mentioned below) acted in the same way.   It therefore seems a fair conclusion that type 953 contains Flexner group antigen and should be classed as a type of *Shigella flexneri* in continuation of the six types listed by Boyd (1938, 1939-40).

Strains 2·193 and 2·694 were sent to Lt.-Col. R. F. Bridges at the Standards Laboratory, Oxford, with a note that they were believed to be a new Flexner

type, and he reported : " I am sure that you will be interested to learn that strains 2·193 and 2·694 are, I think, identical with the strain 953 which I brought with me from India in 1937. The original strain was isolated by D. T. M. Large in Quetta about 1934. Subsequently I received several other strains of the same type, and I have notes of isolations in Rawalpindi and Kohat, so it seems that this type was widespread about the N.W. frontier of India, but certainly not common ". Bensted (1939) mentions this type as having been identified three times in three years out of an average number of 2000 strains of dysentery bacilli n year.

Ewing (personal communication, 1945) reports that the antigenic relationship of strain 2·193 to Boyd's type V, P.143, and to the Flexner group has been confirmed by Wheeler in the United States, and none of us is able to agree with Noter (1944, 1945) that 2·193 should be regarded as a new type of *Shigella alkalescens* of different antigenic type from the classical and the de Assis types.

In his report on 2·193 and 2·694, Bridges mentioned that cultures of strains of the 953 type had shown two colony variants, one of which was partially resistant to agglutination, but the subculture of strain 953 which he sent consisted of uniformly agglutinable colonies. Strains 2·193 and 2·694 were re-examined six months later, and it was found that the majority of colonies on plain nutrient agar plates were of a larger and flatter type than those originally seen, and these colonies had an oily sheen. Such colonies, and subcultures from them, were partially or totally resistant to agglutination unless first treated by boiling or with chloroform, just as was described by Archer (1942) in the case of the opaque colony phase of the classical *Shigella alkalescens*. This phenomenon has apparently not been observed by Noter.

Type 1296/7 (provisional *Shigella flexneri* type VIII)

Two strains of this type were received from the Central Pathological Laboratory, Middle East Force, in 1944 and a third in 1945. It also is a rare type, and was identified six times among a group of 109 atypical mannitol-fermenting dysentery bacilli studied at the Central Pathological Laboratory. These were a sample of 800 such strains which accounted for 3·34 per cent. of dysentery bacilli isolated in the Middle East theatre from August 1940 to June 1943. This type is mentioned by Boyd (1946). These strains appeared to represent a new antigenic type, and rabbits inoculated with suspensions of killed bacilli developed high-titre Flexner group-phase agglutinins for Y and 103 B. This Flexner group agglutinin was removed by absorption with any of the three strains.

General characters of the new types

All strains examined were non-motile, non-sporing, non-capsulated, Gram-negative, aerobic bacilli, yielding smooth, hemispherical, translucent colonies of 1·3 mm. diameter on nutrient agar after 24 hours at 37° C. All were methyl red- and indole-positive and Voges-Proskauer- and citrato-negative. All fermented glucose and mannitol within 24 hours, producing acid but no gas, and they failed to ferment lactose, sucrose or salicin within 14 days. Dulcitol was fermented by strain 953 after 2 days, but only slight transient acidity was produced by 2·193 and 2·694. Strains of type 1296/7 all failed to ferment dulcitol. Arabinose, rhamnose and maltose were fermented by all three strains of type 953. Two strains of type 1296/7 formed acid in rhamnose after 5 days, but all three strains failed to ferment arabinose or maltose within 14 days.

None of the strains showed significant agglutination with sera of the six recognised Flexner types or the six Boyd types (except type 953 with Boyd V, P.143, as already noted), with sera for *Shigella sonnei*, *shigæ* or *schmitzi*, with

sera for the Sachs non-mannitol-fermenting types, or with Salmonella O sera including factors up to XXX. None of the strains was agglutinated by sera containing the alpha agglutinin described by Stamp and Stone (1943-44), and sera prepared from the new Shigellas did not agglutinate suspensions of alpha-containing strains of paracolon bacilli.

Summary

Three strains of each of two new types of dysentery bacilli have been examined. With each type, inoculated rabbits developed high-titre agglutinin for Flexner group-phase bacilli as well as specific agglutinin, and absorption of these sera by strains of homologous type resulted in the removal of both homologous and Flexner group agglutinin.

It is suggested that the type represented by strains 953, 2-193 and 2-694 should be designated *Shigella flexneri*, type VII, and the type represented by strains 1296/7, 1320 and 356 *Shigella flexneri*, type VIII, in continuation of the series described by Boyd (1939-40).

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576 . 8 . 095 . 312 : 576 . 851 . 214 (*Str. hæmolyticus*)

THE METABOLISM OF GLUTAMINE BY MICRO-ORGANISMS IN RELATION TO THE NEED BY HÆMOLYTIC STREPTOCOCCI FOR ADDED GLUTAMINE IN GROWTH

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Unit for Research in Cell Metabolism (M.R.C.), Department of Biochemistry, The University, Sheffield

Several strains of β-hæmolytic streptococci require about $5 \times 10^{-6}M$ glutamine for half-maximal growth (McIlwain *et al.,* 1939) ; there is thus ample for their growth in tissue fluids, which are about $5 \times 10^{-4}M$ with respect to glutamine (Archibald, 1945). During streptococcal growth in media containing defined quantities of glutamine, the substance disappeared from the media and a comparable quantity was not found in the harvested cells. Washed suspensions of the streptococci did not cause any change in glutamine when that was added alone, but when it was added together with constituents of the growth-medium, one of these—glucose—caused the organisms to decompose glutamine. The decomposition proceeded at the rate of 1-3 μmol./mg. dry wt./hr. and yielded glutamic acid and ammonia. It has so far only been observed while the streptococci are reacting with glucose, which they convert into lactic acid. Glutamine stimulated this process, for example from a rate of 15 to one of 28 μmol. acid/mg. dry wt. of organisms/hr.

To assess the significance of this process in relation to the need of the streptococci for added glutamine in growth, the behaviour of organisms not needing pre formed glutamine has been compared with that of the streptococci Staphylococci could synthesise glutamine from ammonium glutamate in the presence of glucose Yeasts (*Saccharomyces cerevisiae* and *S ellipsoideus*), like the streptococci, decomposed glutamine during their metabolism of glucose, but were also capable of synthesising it from ammonium glutamate *Proteus morganii* decomposed glutamine independently of glucose metabolism by an enzyme which was easily extracted by grinding or autolysing the cells This appeared to be a simple ' glutaminase , in contra distinction to the more complex streptococcal system, which has not yet been extracted from the organisms The exacting β haemolytic streptococci are thus the only organisms of these examined which require glutamine in growth, and which, while decomposing it during reaction with glucose, have no power to synthesise it

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CHORIONEPITHELIOMA TESTIS

G S W DE SARAM and M V P PIERIS

From the Colombo General Hospital, Ceylon

Clinical summary The patient, a man aet 50, was admitted on 26 7 43 with a swelling in the right inguinal region of 2 years' duration It was hard and tender, and when first noticed was about the size of an almond Within the last 2 months it had increased in size and had become increasingly tender. At operation on 27 7 43 a swelling 5 × 2 inches was found, extending from the external abdominal ring into the right side of the scrotum This consisted in its lower half of a cyst filled with degenerate blood clot intimately adhereot to the cord and to the distal end of a hernial sac which was traced to the external abdominal ring Sac, cord and cyst were all excised The right testicle was absent from the scrotum and had been so from birth

Histological findings The tissue consists almost entirely of blood clot except for one small cellular area The majority of the cells are large and pale, with large irregular single, double or multiple nuclei, each possessing one or more nucleoli and showing peripheral chromatin condensation Interposed irregularly amongst these cells are large syncytial eosinophilic masses with irregular outlines and aggregated pyknotic nuclei No testicular tissue can be made out The section is one of chorionepithelioma A Friedmann test carried out 5 days later was positive, and X ray examination of lungs and skeleton showed no secondary deposits

After history The patient was discharged on 13 8 43, but returned 15 days later with extensive involvement of both lungs (confirmed by X ray) and evidence of deposits in the brain The spleen was enlarged one inch below the costal margin He died on 3 9 43, 38 days after operation

Autopsy The body was much emaciated and the organs were riddled with secondary deposits of purplish red growth The third right costochondral junction contained a secondary deposit 1½ inches in diameter The right pleural

cavity contained 15 oz. of deeply blood-stained fluid and there was fibrinous pleurisy over the lower and posterior aspects of the right lung. Both lungs were densely infiltrated with deposits ranging up to $2\frac{1}{2}$ inches in diameter. One of the masses in the right upper lobe had ruptured into the pleural cavity. A nodule $\frac{3}{4}$ inch in diameter was present in the upper part of the right lobe of the liver. The spleen contained several nodules up to one inch in diameter, and in both kidneys there were several small nodules, the largest $\frac{1}{4}$ inch in diameter. An ulcerated nodule one inch in diameter was present in the jejunum. The cerebrum contained several deposits $\frac{1}{2}$-$1\frac{1}{2}$ inches in diameter, including one in the left occipital pole. The right inguinal region showed no evidence of growth.

Microscopically all the deposits show the structure of a chorionepithelioma, with abundant syncytial masses and Langhan's cells with many mitoses.

Remarks. It is to be assumed that the primary growth, a teratomatous chorionepithelioma, originated in an undescended testicle on the right side. The short post-operative history of 38 days and the presence of secondary deposits in the spleen and jejunum are of interest.

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SHAPE AND MOTILITY OF BACTERIA

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(Plates XLIV-XLIX)

" Thus once again we meet, in these lower forms, the spiral organisation which is so common a feature in plants. The conclusion is unavoidable that this prevalence of spiral structure reflects some underlying vital principle that is common to the whole of the plant kingdom " (Astbury and Preston, 1940).

Many kinds of bacteria, usually described as rods, are helices or " spirals ", and the appendages of such bacteria, usually called flagella, are a result of motility and not motile organs. Most of the present work on this subject was done on *B. typhosus*, but observations on *B. proteus*, *B. megatherium*, *B. cereus*, *B. subtilis*, *B. fluorescens* and others indicated that this new conception of shape and motility applies widely. For the purposes of this paper " bacteria " means most of the " Eubacteriales ".

Bacterial flagella : stained preparations

The invisibility of flagella with ordinary microscopy has led to the invention of numerous staining methods, satisfactory to their authors, but usually a failure in other hands. Often mucous threads attached to bacteria have been stained and regarded as flagella. Instances are the denial by van Niel (1923-24) of Ellis's claim (1902, 1903-04) that certain sarcinæ possessed flagella, and the supposed flagellation of *B. tularense*, refuted by Hesselbrock and Foshay (1945). Zettnow, inventor of a much used staining method (1899), admitted later (1918b) that he could not always differentiate between " mucous threads " and flagella, especially when mucous threads showed a wavy appearance, and added that such structures should only be regarded as flagella when motility had first been established ! Hinterberger (1921) similarly warned against confusing what he called " Myzele " with true flagella, but his basis for

FIG. 1.—Drop of broth culture drying up: flagellar appendages swept across field in current set up by drying process. ×2000.

FIG. 2.—Stained typhoid bacilli with flagellar appendages: windswept appearance. ×1000.

FIG. 3.—Stained typhoid bacilli with flagellar appendages, showing large number of wavy threads and a gap between threads and bacilli. ×1000.

FIG. 4.—Stained typhoid bacillus with two flagellar appendages. ×1000.

FIG. 5.—Typhoid bacilli in gum solution: thickened flagellar appendages, one or two per individual. ×1000.

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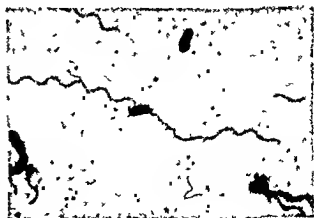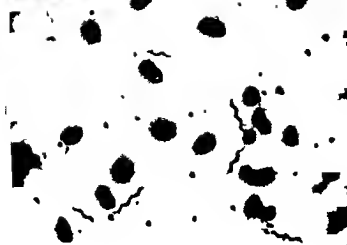
Firstly the surprisingly small number of flagellar appendages seen in viscous colloid solutions cannot be due to a large number having become plaited together, because normal motile bacteria swimming in thin watery solutions ordinarily show exactly the same small number of flagellar appendages. Secondly the particularly thick appearance of the appendages is due to a coating of gelatin or gum which precipitates from the solution on to their surface. Watching a preparation for a couple of days showed that eventually the coating breaks up into small granules which then slide up and down the spiral appendages and finally fly off, leaving behind very thin wavy threads (fig. 6).

This coating process is curiously similar to that observed by me (Pijper, 1938) in H agglutination of *B. typhosus*. Here the process is slower and its stages can easily be followed and filmed (1941a) with sunlight dark-ground microscopy. First granules appear on the appendages (fig. 7). These are probably immuno globulins. They then increase in number and coalesce into a continuous sheath (fig. 8). Here too the sheath eventually breaks up into granules again (fig. 9). Similar findings with agglutinating sera were reported with the electron microscope by Mudd and Anderson (1941). There is thus close similarity between the appearance of motile bacteria in these colloid viscous solutions and typhoid bacilli in H serum. Obviously neither picture can be regarded as reflecting normal conditions. The thickening seen is not due to plaiting but to a precipitate.

Bacterial flagella : visibility in thin watery solutions

The sunlight dark-ground technique (Pijper, 1931-32, 1938, 1940) showed that motile bacteria such as *Bact. typhosum*, *B. proteus*, *B. megatherium* and others, usually described as peritrichous, in water or broth ordinarily present themselves as swimming with a long tail (fig. 10). It really is a long-drawn-out spiral, as becomes obvious when speed is suddenly reduced (fig. 11). Occasionally, and usually at rest, the tail untwists into two spirals (fig. 12). This change is reversible, the tail reconstituting itself when movement is resumed. A more important phenomenon is that the tail, or the two flagella, can untwist into a large number of very thin wavy threads, which never impart movement to the bacterium and after a time drift away (1938). This final untwisting is irreversible. These wavy thin threads are visible with sunlight dark-ground microscopy, but very difficult to photograph : fig. 13 does not do them justice. Fig. 14 is a diagrammatic drawing of this irreversible untwisting process which is the explanation of the incongruities mentioned. It is clear now that during active life there are only one or two flagellar appendages per cell, and it is these appendages that are thickened by the precipitate of gum, gelatin or globulins referred to above. During staining processes, if one disregards for a moment " borrowed " flagella due to the " receding tide ", the appendages may become fixed either while they still form a tail (or two flagella), or later, while final untwisting is taking place. The result is a bacterium with one or two wavy appendages, like fig. 4, or in the later stages a bacterium surrounded by a more or less thick fleece of wavy threads, like fig. 3. In many cases these thin wavy threads are separated by a short distance from the cell.

Bacterial flagella : the electron microscope

Electron microscope pictures of various motile bacteria made by Piekarski and Ruska (1939) showed bacteria surrounded by varying numbers of long thin wavy threads, not definitely attached to the bacteria. Obviously these threads are the same as those seen by me as the result of the final untwisting of tails and flagella (fig. 14).

Mudd and Anderson (1941) published similar pictures of *E. typhosa* and *B. subtilis*. Further pictures of *B. subtilis* by Mudd, Polevitzky, Anderson and Chambers (1941), where some of these threads are seen in contact with the cell wall, evidently do not prove continuity with that structure. Discussing similar pictures of *V. schuylkiliensis*, Mudd, Polevitzky and Anderson (1942) pointed out that sometimes the wavy threads seemed continuous with the protoplasm but that no basal granule was visible. Knaysi (1944, p. 89) thought that in one picture of *V. choleræ* by Mudd and Anderson (1942) there was a suggestion of a basal granule (which the authors themselves did not mention), but added that no definite conclusion could be drawn. In a recent review Mudd and Anderson (1944) present numerous pictures of bacteria with these wavy threads lying about, some of them seemingly traversing the cell wall but in many more nothing of the kind is seen. Recent electron microscope pictures by Williams and Wyckoff (1945) of motile bacteria again show thin wavy threads in no way connected with the cells. In electron microscope preparations it would indeed be strange if the thin wavy threads never became superimposed on bacterial bodies.

It appears then that sunlight dark-ground microscopy satisfactorily explains the incongruities of the other methods of studying flagella. Nothing so far has become known about the morphology of flagella which has not been visible in sunlight dark-ground, and this method must be accepted as the safest guide because it excludes artefacts.

The thin wavy threads which can be made visible by various methods as surrounding motile bacteria and which during motility usually form a tail and sometimes two spiral flagella, have so far been regarded as motile organs without close enquiry into mechanics or function. The argument that all motile bacteria show such appendages and non-motile ones do not, and that therefore these appendages must be motile organs, is bad logic, but it has been accepted since Loeffler's day. Dubos (p. 47) at the end of a very exhaustive review concluded that " much remains to be learned of the occurrence and importance of flagellation in the bacterial world ".

Structure of bacteria

Flagellar appendages must be connected in some way with the cell. Recently a good deal more has become known of the structure of bacteria, chiefly through work of Knaysi (1938, 1944), and this has been amplified by the studies of Mudd and his co-workers with the electron microscope. The terms ecto- and endoplasm have lost meaning : the central part of the bacterial cell is taken up by protoplasm or cytoplasm. This is mostly a rather thin colloid fluid, but

PLATE XLV

FIG. 6.—Typhoid bacilli in gum solution : thickening of flagellar appendages has become granular. ×1000.

FIG. 7.—Typhoid bacilli in H serum : granular deposit on wavy tail. ×1000.

FIG. 8.—Typhoid bacilli in H serum : coating of flagellar appendages. ×1500.

FIG. 9.—Typhoid bacilli in H serum : coating of flagellar appendages breaking up into granules. ×600.

FIG. 10.—Typhoid bacillus with tail, in broth, swimming fast. ×2500.

FIG. 11.—Typhoid bacillus with tail, in broth, slowing down. ×1000.

FIG. 12.—Typhoid bacillus in broth, with tail untwisted. ×2000.

FIG. 13.—Typhoid bacilli in broth ; flagellar appendages, untwisted into thin wavy threads, floating away. ×2500.

SHAPE AND MOTILITY OF BACTERIA .
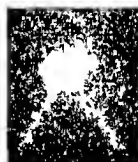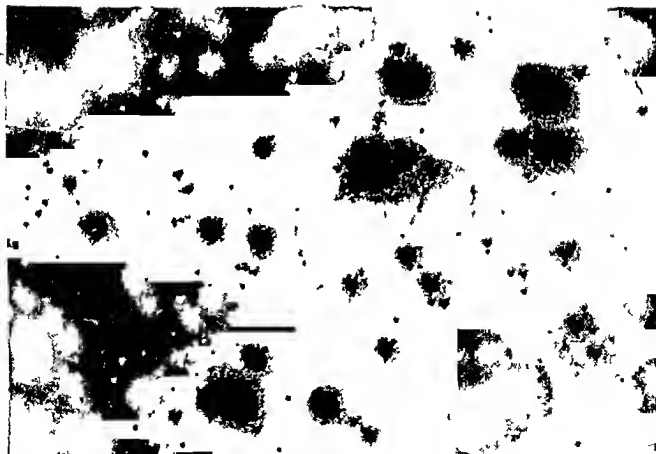
it becomes condensed towards the periphery to such a degree that a definite cytoplasmic membrane is formed, which stains with the usual bacterial stains and shows up brightly in dark-ground (fig. 15), the central more watery region of the cell remaining dark. Fig. 16 is a diagrammatic drawing of the bacterial cell, based on photo-micrographs of typhoid bacilli. "B" is the cytoplasm, becoming more condensed towards the periphery. Immediately surrounding this lies the thin cell wall proper "A", of non-protoplasmic material. It is rigid and elastic and keeps the bacterium in shape, acting as a sort of exo-skeleton. It can be shown up by plasmolysis, which makes the cytoplasm recede (fig. 17), and also by certain stain-ing processes (Knaysi, 1944, p. 39). An excellent way of demonstrating its existence and nature is by letting a bacterio-phage destroy the cytoplasmic layer (Pijper, 1941b). This reveals the cell wall as a thin membrane (fig. 18), which sometimes demonstrates its elasticity by being blown out into bubbles when the pressure inside the cell rises (Pijper, 1945). Electron microscopy has also demonstrated this cell wall in cases where the cytoplasm had retracted from it during the drying process (Mudd, Polevitzky, Anderson and Kast, 1942), and especially clearly when the cells had first been subjected to ultra-sonic destruction (Mudd, *et al.*, 1941). The chemical structure of the cell wall is not certain, but it is probably a complex carbohydrate. At any rate it separates off the live substance of the cell from the outer world.

This rigid cell wall is surrounded by what Knaysi (p. 44) calls the slime layer (fig. 16, "C"), which is present on the surface of most if not all bacteria (Henrici, 1939, p. 121; Knaysi, 1944, p. 44). When very thick it is called a capsule, as in pneumococci, although some authors (Dubos, p. 37) prefer this term for all bacteria. It provides what Stephenson (1943, p. 70) has called the slimes and gums of bacteria and is probably similar to the slime layer known in algae. In some cases its quantity can be increased by growing bacteria at a low temperature in the presence of glucose (Morgan and Beckwith, 1939a, 1939b). Its origin is obscure; it may be a gelatinised outer layer of the cell wall, a secretion product, a product of metabolism or a protective organ. It cannot itself have metabolic properties, as it consists of complex polysaccharides which occur in the natural state as long chain polymers. These vary in structure with species and even with strain, and this accounts for a degree of immunological specificity. They are very viscous, and in some cases can be drawn out to a length of several cm. (Knaysi, 1944, p. 44); this is due to the curious molecular structure. It has been shown (Dubos, p. 41) that the slime layer can be washed off or made to disappear by enzymatic depolymerisation without affecting the viability of the bacteria. It cannot be regarded as a live substance, although it contains or represents species-specific substances, which however are usually present in other parts of the body as well.

The relationship of flagellar appendages to the structures described (cytoplasmic membrane, cell wall and slime layer) has received little attention. Zettnow (1918*a*) could not follow his flagella further than what he called the ectoplasm. There is no morphological evidence that flagellar appendages ever reach the cytoplasm, nor is there a basal granule.

Fuhrmann claimed to have seen a bundle of twisted flagella go through the cell wall at the polar end of *Sp. volutans* and become attached to a blepharoplast. This was completely refuted by Meyer (1912, p. 117) and in any case Fuhrmann offers only drawings to back his opinion ; his photomicrographs merely show a number of thin wavy threads lying about. It is also known that bacteriophage action destroys the cell but leaves flagella intact (Luria, 1943). Direct attachment to the cell wall has not been demonstrated and, even in the best photomicrographs of stained preparations, there is often a gap between flagella and cell wall (fig. 3). Nevertheless there is a belief—and Dubos (p. 44) still mentions it as a possibility—that flagella grow from the cytoplasm through holes in the cell wall. Now it is shown above that flagella during activity form a twisted tail (fig. 10), which is a bundle of very thin wavy threads, sometimes numerous enough to form a fleece around the bacterium if they unwind completely (figs. 3, 13 and 14). During activity there would then be an arrangement diagrammatically represented in fig. 19. Apart from the biological novelty of having a rigid cell wall pierced by such a number of motile threads, all tightly fitted, this would cause a very strained position mechanically. Motility of each thread would start inside the cell and be transmitted along the rectangular bend after the threads pierce the cell wall through a tightly fitting hole. What would happen at a sudden reversal of motion is still more difficult to understand.

The only place left where flagellar appendages can originate is the slime layer, as illustrated in fig. 16, " D ". This would explain why in stained preparations there is so frequently a gap between cell wall and flagella. It also fits in with the observations that flagella and slime layer have similar staining reactions and that neither is essential for the life of the cell. I have shown (Pijper, 1938, 1941*a*) that the specific precipitate which descends on the flagella as the first stage in H agglutination descends on the slime layer as well. Flagella and slime layer therefore must have much in common.

Flagella in animals

In contrast to bacterial flagella, animal flagella, especially those of protozoa, are easily seen in action by ordinary microscopic methods ; they stain readily and are much larger. As stated by Gray (1928, p. 2), in the protozoal world flagella are motile organs of considerable length and each flagellum is a separate vibratile unit with its own blepharoplast. As protozoal flagella always penetrate into the cell and there link-up with a blepharoplast, they can be regarded as part of the protoplasm. The cell wall of protozoa being quite different from the cell wall of bacteria lends itself to such an arrangement. Animal flagella also do not become detached so easily as bacterial flagella.

Analogy of animal and bacterial flagella

The superficial similarity of animal and bacterial flagella has proved too much for most investigators. Similarity in appearance, however, does not

SHAPE AND MOTILITY OF BACTERIA

establish analogy. The assumed analogy made it appear superfluous to look for other sources of motility in bacteria. Thus efforts were concentrated on making bacterial flagella visible by staining, ignoring physiological and morphological aspects. My own early efforts to make bacterial flagella visible by dark-ground microscopy (Pijper, 1930) led me to the employment of the most brilliant light source available, which was sunlight. This resulted in blurring of the bacterial bodies (fig. 11). Preoccupation with flagella in the shape of tails and the fact that faster bacteria showed longer tails made me overlook the movement of the bacterial body, which moreover was blurred by the bright sunlight.

Effect of methyl cellulose on bacterial motility

Methylcellulose * is a non-toxic substance, readily soluble in ice-cold water, forming clear solutions which become gels on heating. It has been used by Marsland (1943) for slowing down the inconveniently rapid motility of Paramecium.

At the suggestion of Professor R. S. Breed, New York State Agricultural Experiment Station, who also supplied me with a quantity of the material, I investigated its effect on the motility of bacteria, with striking results. A solution of suitable strength provided just those conditions previous authors had aimed at with gelatin and gum solutions, namely a medium which merely through viscosity slowed down movement of otherwise very fast bacteria. This colloid substance, then, did not precipitate on flagella and bacterial bodies to any harmful extent for an appreciable time. The appearances became like a slow motion film of fast bacteria in broth or water. Exact data for suitable solutions are difficult to give, as the effect varies with temperature, method of preparation and degree of motility of bacteria. A solution of $1\cdot5$ to 2 per cent. methocel of the 4000 centipoise type in saline answers well. The technique is to prepare graded solutions of methyl cellulose and to try each of them, placing a drop from a 5 mm. platinum loop on a slide and gently rubbing into it a drop from a 1 mm. platinum loop of a very motile broth culture. A coverslip is quickly put on and the preparation sealed.

In successful preparations one sees, in sunlight dark-ground, bacteria with long tails, perhaps a little thicker than in broth but otherwise identical, swimming very slowly and with a very peculiar and striking movement of their bodies. The bodies exhibit a gyratory and at the same time an undulatory movement. They move in spiral fashion, bending their bodies and curving their walls. It becomes obvious that these bacteria are not rod- but spiral-shaped. (It would be pedantically correct to speak of " helix " and " helicoidal ".) With a binocular microscope, allowing stereoscopic vision, one is particularly struck by the gracefulness of the movement, which is not just a snake-like undulation, but a gyratory undulatory movement in space. The cell wall shows surprising pliability and elasticity considering its rigid nature. The longest bacteria show up best, but the smaller and even the very short can easily be followed in their contortions.

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* It is sold as " Methocel " by the Dow Chemical Company, Midland, Michigan, in four types of viscosity, and I express my gratitude to this firm for supplying me with material for experimental purposes.

Fig. 20 shows a short section from a 16 mm. cinemicrographic film made from such a preparation. It illustrates the spiral contortions quite well, but of course, represents very inadequately what the motion picture shows. The sight of so many supposedly rod-shaped bacteria, all undulating and gyrating in spiral fashion, forces one to the conclusion that the spiral shape is their normal shape, that these undulatory and gyratory movements are quite sufficient to propel them and that it is unnecessary to look for special motor organs. One realises that this is the form of locomotion which bacteria normally use. That this has so far escaped attention is largely due to the fact that in fast bacteria in thin watery solutions the spiral shape of the body becomes rather drawn out, thus approaching the shape of a rod. Another reason is that attention has always been focussed on motile organs, as in my own case where I blurred the bodies of the bacteria in order to make the tails visible.

A curious phenomenon in methylcellulose, which assists close observation, is that many bacteria swim in small circles. The straight line which bacteria in thin watery solutions seem to follow is in essence a large circle. The viscosity of methylcellulose solution, by impeding fast movement emphasises the deviation from a straight line by reducing the size of the circle.

As soon as one accepts the idea that the gyratory undulatory movement of the bacillary bodies, which becomes so manifest in methylcellulose solutions, is the mechanism which propels bacteria, one realises that appendages such as tails and flagella need no longer be looked upon as motile organs.

Spiral movement of bacteria in broth

Methylcellulose solutions having pointed the way, other methods were looked for to bring out the gyratory undulatory movement and thus the spiral shape of bacteria. For this purpose a " slow motion " cinemicrographic 16 mm. film was made of bacteria in broth which moved so fast that in sunlight dark-ground no gyratory undulatory movement could be made out. The slow motion film, having reduced the speed approximately four times, showed on the screen bacteria which moved in typical spiral fashion, exactly like those in methylcellulose solutions.

Even without slow motion cinemicrography the spiral nature of the motion can sometimes be seen. Fig. 22 is an instantaneous photomicrograph of a typhoid bacillus swimming at high speed in broth. To the discerning eye there is a definite twist in its body.

Further, *B. typhosus, B. megatherium, B. proteus* and *B. subtilis* were grown at room temperature in winter time (about 17° C. in Pretoria), which reduced their speed very much as compared with the same bacteria grown in the incubator. All four at this lower speed showed typical undulating gyratory movement and spiral

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FIG. 20.—Consecutive pictures of 16 mm cinemicrographic film : typhoid bacillus in methylcellulose solution, showing undulating gyratory movement of body. × 1000.

FIG. 21.—As fig. 20, showing shorter bacillus. × 600

shape. An added advantage was that the low temperature reduced fission, so that much longer forms were present which showed the phenomenon particularly well.

Shape of dead bacteria

The conventional description of bacteria as rods is due to their appearance in killed, fixed and stained preparations from ordinary cultures. It was stated above that the faster a bacterium swims, the more drawn out its spiral shape becomes, thus tending to resemble a rod. Perhaps *rigor mortis* emphasises this shape. It remains curious that in bacteriological literature "slightly curved rods" are so often mentioned and depicted. Yet it is surprising how often in text-books and other publications one comes across pictures of definitely spiral-shaped bacteria where the text speaks of rods. The point is not that one comes across these spiral forms occasionally, but that one meets them so often, once one has learned to look for them. It is not enough to say they are "slightly curved rods", for they are definitely elongated S-shaped figures, which of course means projections of spirals. Occasionally spiral shapes have been noticed but their meaning was overlooked, as in the observation of Knaysi (1944, p. 20) that "helicoidal cells are frequently seen in such typically rod-like organisms as *B. megatherium*". Similarly Gillespie and Rettger (1939) made nothing of what they called the "twisting" cells of *B. megatherium*, which they even saw winding themselves together, and Miessner, Meyn and Schoop (1931) saw "twisted cells, wound together" in *Clostridium novyi*, but again the meaning escaped them.

The spiral shape is particularly common in bacteria that have died, or at least stopped moving, in methylcellulose solutions. Movement here is slower and the spiral broader than in broth, and the bacterium remains fixed in its shape in the viscous solution. By leaving motile bacteria of various kinds in methylcellulose solutions under the microscope until movement stops, one gets pictures like figs. 23-32. In nearly all of these the spiral shape is obvious, though the longer forms show it more clearly than the short; but there are many short forms that are obviously part of a spiral.

Considering then both the shape during motility and the shape when lying still in suitable methylcellulose solutions, which cannot be regarded as an abnormal medium, one must conclude that the normal shape of a motile bacterium is not rod-like but is that of a spiral or helix (fig. 33). To how many bacterial species this conception applies is difficult to say at the moment. It does not seem excluded that even those that are non-motile nowadays still preserve an indication of a previous more active period and shape.

Motility and flagella

Gray (p. 1) said that when a cell wants to do mechanical work and cannot undergo marked changes in form it has to use cilia or flagella. As here shown, motile bacteria can do their mechanical work by changing their form and therefore do not need flagella.

I have shown that in motile bacteria such as *B. typhosus, B. proteus, B. megatherium, B. subtilis* and others, during activity, there is attached to the body a long tail (Pijper, 1930, 1931-32, 1938, 1940). If this tail really constituted the motile apparatus, one would expect it always to be visible, other

conditions being the same. This is not the case. During the many years I have studied bacterial tails in sunlight dark-ground there were always times when motility was excellent but tails almost completely absent or only faintly visible. This was usually followed by a period when the same bacteria showed well developed tails. I have no full explanation of this phenomenon of waxing and waning tails, but I know that the medium is responsible to a certain extent. Batches of broth differ in their production of good tails, older batches usually being more satisfactory. Since efforts to influence tail changes in the medium gave uncertain results, I had to content myself with looking for a batch of broth in which good tails appeared.

The assumption that the whole flagellar apparatus which forms the tail is not an active motile organ but a non-essential product of motility suggests a simple explanation for the existence of very motile bacteria without tails. The assumption also explains the frequent failure in staining flagella of motile bacteria.

That flagella are not essential for motility is commonly accepted for the Myxobacteriales, which creep or crawl without flagella. This was noticed early by Vahle (1909-10) with *Myxococcus ruber* and later with *Myxococcus xanthus* by Beebe (1941), who thought that motion here might be caused by " asymmetric excretion of slime ". Knaysi (1938, 1944, p. 84) was probably nearer the truth when he suggested about sulphur bacteria that they " creep slowly " as a result of " waves of contraction which cause periodic alterations in the form of the cells ". In these cases again the authors unwittingly show pictures of S-shaped bacteria, like my figs. 23-33. In the case of *Cytophaga columnaris*, another Myxobacterium, Garnjobst (1945) described a creeping movement, which was occasionally rapid and which could reverse. In water one end of a cell showed peculiar rotary or waving movements, which again seemed to him to require flagella, but these were never found. Incidentally, in blue-green algæ a similar type of motion occurs.

These non-flagellated forms, then, execute somatic movements at low speed, rather similar to those of my normally fast-swimming bacteria when their speed is damped down by methylcellulose. It therefore appears that it is the faster motion which produces tails or flagella, and I have always noticed that the faster my motile bacteria moved the longer their tails became.

The argument that it is still the tail that provides the motion and, in doing so, makes the body rotate and undulate, does not bear analysis. The thin feeble tail can hardly have enough power to bend and curve the rigid cell wall to the extent seen and photographed by me (figs. 20 and 21). Also, sudden and repeated reversal of motion is a feature of bacterial motility. I have always been struck by the rapidity with which this takes place, and it is hardly thinkable that a whole flagellar apparatus consisting of numbers of actively waving threads twisted into a tail can swing over rapidly enough to the other end of the bacterium and there start pushing again. Also, from the beginning I have stated (Pijper, 1930) that a bacterium can swing its body through an angle of 180°, so that what was the front becomes the rear, whilst the visible tail remains undisturbed and the bacterium goes on swimming in the same direction as before, with just its poles reversed. Both these phenomena, the sudden reversals and the polar

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PLATE XLVIII

FIG. 22.—Instantaneous photomicrograph of fast-swimming typhoid bacillus, showing spirillar twist of body.. ×1500.

FIGS. 23-32.—Motile bacteria having come to rest in methylcellulose solution, showing spiral shapes. ×600-1500.

turn-abouts, are difficult to explain on the basis of a tail as motor-organ, consisting of a number of active wavy threads, all firmly attached to some part of the body of the bacterium. If, however, the tail and its constituent threads are merely passive appendages, their explanation becomes simple.

Mechanical arguments against flagella as motile organs

A motile bacterium which has just undergone fission may go on swimming in a straight line, corresponding to the coinciding long axis of the resulting two bacteria. One may assume that the common slime layer holds them together to a certain extent. Very often, however, one meets with structures like fig 36 swimming along the line indicated by the long arrow, whilst the segment in front revolves round this line as indicated. With a tail as motive force pushing from behind, a slime layer could hardly prevent the combination from folding up If however the motive force is situated in the bodies of the bacteria the combination would be stable, as indeed it is. As pointed out by Lowndes (1941), an elongated body driven in a certain direction by an outer motive force will be in its most stable position when its long axis is at right angles to the direction in which it moves. Motile bacteria are nearly always found with their long axis in the direction of movement, which is to be expected if the body is propelled by gyratory undulating movements of the body itself.

Physiological considerations

If the thin wavy threads which build up the tail or flagella were really motor organs, they should get their energy from somewhere. They do not generate it themselves, as the shape of the tail indicates, t because it does not show an increasing amplitude and wave-length from origin to tip. The thinness of the constituent threads also precludes this ; Mudd and Anderson (1941) found them to be about 300 Å. units thick. If they just transmitted energy, deriving it from their origin, which is the slime layer, the slime layer would have to act as a continuous source of energy of a very particular kind. Its nature makes this impossible. The gyratory undulating movement of the bacterial body being quite sufficient to propel the bacterium, it would also be uneconomical to introduce an additional source of energy. This brings one back to the conception that flagella are better regarded as appendages produced from the slime layer by the spirillar movement of the body. In simple words, the dog wags the tail and not the tail the dog.

The motive force required to propel bacteria cannot be great Their specific gravity is very near to that of water. There can hardly be a more " streamlined " body than a bacterium with a tail (fig. 10).

Stability of direction must be greatly assisted by the tail and, once set in motion, very little energy will be needed to keep the streamlined structure going. A good deal of "coasting " is probably done, similar to the gliding movement of goldfish in a bowl, after one short wave of muscular contractions has passed over its body. Naturally when a bacterium swerves the tail will follow in a curve,

creating the illusion that it steers the bacterium. Used as one is to the idea of steering and propelling gear at the rear of a body moving through water, it takes an effort to look upon the bacterial tail, not as a propelling screw and rudder combined, but as at best a steadying though wholly passive appendage. Such a conception brings bacteria into line with other aquatic creatures.

Origin of flagellar appendages

The very viscous anisotropic polysaccharide material of the bacterial slime layer consists of long-chain molecules which readily combine into longer micellæ (Dubos, 1945, p. 38; Knaysi, 1944, p. 44). The gyratory undulating movement of their bodies which propels bacteria subjects this material to a twisting influence. The slimy material hangs loosely round the body and is not firmly attached to the cell wall. The effect of rotation combined with forward movement is that the slime layer is drawn out into the tails described (fig. 10). The slime, when the bacterium swims fast, is in effect subjected to spinning, like wool on a spinning wheel, and the result is similar. The dark space between body and tail, often noticeable in photomicrographs, is due to the slime layer at that spot not being sufficiently condensed to become visible in dark ground. In the tightly twisted tail conditions are different. These points are diagrammatically illustrated in fig. 37.

Factors such as quantity and quality of the slimy material decide how long and how thick the tail may become. The spiral shape of the tail is of course derived from the gyratory movement of the bacterial body and so the spiral becomes broader as motion slows down (fig. 11). An increase in production or in density of the slimy material will affect the length and visibility of the tail. Good motility without a properly visible tail means that there is not enough slimy material or that it is not sufficiently dense. The development of a tail is partly a question of bacterial metabolism. The observation of Leifson (1931) that bacteria developing from a spore show flagella which increase in length with the passing of time, merely demonstrates that more slimy material becomes available with time.

I have mentioned that batches of broth differ in the readiness with which tails become visible in cultures. The explanation is that batches of broth differ in the ease with which bacteria can produce a satisfactory slime layer from the materials provided in the broth. This question was taken a step further by growing the same very motile bacteria in good broth and in peptone water. Although very good motility was preserved in peptone water, tails as a rule were absent or very poorly developed as compared with the control in broth. Different peptones differ in their quantitative effect, but the phenomenon persists. There must be something present in good broth which provides bacteria with a good slime layer and which is absent in peptone water.

Conversely, by adding gum or gelatin to broth, particularly thick tails can be produced by the precipitation of the colloid material on the normal slime layer. Gum and gelatin usually cover bacterial bodies and appendages so

PLATE XLIX

FIG. 33.—Perspective drawing of model of motile bacterium, showing spiral shape.

FIGS. 34 and 35.—Dividing typhoid bacilli, lying still; spiral shapes well adapted to "slipping". ×1000.

FIG. 36.—Diagram of divided typhoid bacilli, moving fast in direction of arrow and not folding up.

FIG. 37.—Diagram of motile bacterium with tail: transition of slime layer into tail, explaining dark spot between body and tail.

FIG. 38.—Diagram of sudden reversal in motile bacterium, showing transition of tail into slime layer and back again at other end.

FIG. 39.—Diagram of motile bacterium turning half a somersault : the tail remains in place.

SHAPE AND MOTILITY OF BACTERIA

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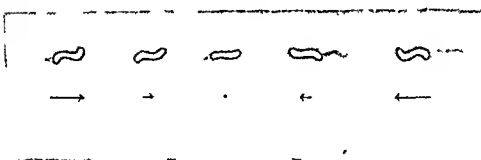
38
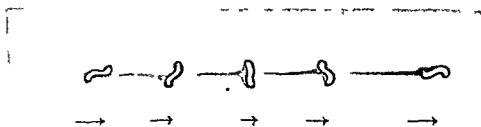
39

thickly that the bacterial bodies have difficulty in moving at all (fig. 5). Methylcellulose in suitable solution is more gentle in its effect. Solutions a little stronger than what is required for just slowing down motility provide a slight coating of the slime layer—enough to make tails more readily visible without paralysing motility. It may be that the molecular structure of methylcellulose resembles that of the slime layer and that the two substances blend easily.

Repeated stopping and starting is a common feature of bacterial movement. During this the slime of the tail may flow back round the bacterial body, with complete disappearance of the tail, and be twisted into a new tail again when movement is resumed. It may also momentarily show broader windings (fig. 11) and then resume the elongated shape.

Another feature of bacterial movement is sudden reversal of direction. What happens is that, at the moment of stopping, the slime of the tail flows back and surrounds the bacterial body. Then the body starts gyrating and undulating in the opposite direction and the slime layer is twisted into a tail at the other end, as illustrated in fig. 37. This is probably the basis for the observations of Hutchinson and McCracken (1943), who described a tail at either end of a "rod-shaped organism of undetermined identity", but during activity in dark-ground the film recorded only one tail.

A bacterium may, as mentioned, keep on swimming in the same direction whilst exhibiting a polar shift, the anterior pole becoming the posterior whilst the tail remains in its old place. This means that the bacillary body can turn a half somersault inside its slime layer with such rapidity that it does not carry the slime layer with it. The phenomenon exemplifies the very loose attachment of the slime layer to the cell wall and is illustrated in fig. 39. It is hardly explainable on the basis of the appendages being motile organs.

A moving bacterium, then, must be visualised as possessing a slime layer which undergoes twisting all the time. Its long micellæ built of long-chain molecules are well adapted to sliding past one another and becoming twisted threads. The slime layer is turned into strands of polysaccharides which have a tendency towards spiral shape. Full activity gathers them together into a tail, which may occasionally split into two flagella (fig. 12) and then reunite again, the material being very plastic and probably nearly perfectly elastic.

It will depend on the nature and quantity of the polysaccharides, in combination with many other factors, what formation the mass of slime material will eventually assume. Among these factors are the varying conditions of microbial life, together with surface-tension, centrifugal forces and friction by the surrounding fluid. The combination of all these features will decide the number and localisation of appendages that will untwist themselves from the tangled mass of more or less pre-formed wavy threads surrounding the bacterial body. With short spirals such as vibrios they will more easily remain together and thus cause a mono- or lophotrichous appearance. Surface tension will be lowest at places of sharpest curvature, which will usually be at or near the poles. With an elongated undulating gyrating body, where large areas come into play at the same time, a number of threads may be whirled off, creating the impression of peritrichous flagellation (figs. 3, 13 and 14).

The chemical varieties of the slime layer must be very numerous, in keeping with the wide variation in immunological specificity. Physical and chemical properties must be closely interwoven, and so there is room for a good deal of variety in the appearance of the

appendages that will arise. Slight temporary variations in physical structure are enough to explain the different types of "flagellation" so often found in stained preparations. The conception that number and localisation of flagellar appendages are characteristic for species now appears in quite a different light and can only be upheld with a good deal of reservation.

This new conception of the nature of flagella does not detract from their interest. D'Arcy Thompson (1942, p. 71) held that "flagella" are, on account of their dimensions, mostly under the peculiar conditions of a surface layer and therefore a portion of matter in a state *sui generis*, with properties of its own, not quite understandable to us. I suggest that amongst such properties are the forces which make granules of precipitated colloid material such as gum or gelatin run up and down the spiral threads as described above. Similarly it happens that in methylcellulose solutions slightly thickened spirals attached to motionless bacteria keep on executing spiral movements, apparently alternating in clock-wise and anti-clock-wise fashion. Such aimless movements do not imply that there is "life" in the spiral threads and are best explained as the result of stresses and strains in the material, on the lines suggested by D'Arcy Thompson (p. 406).

Mechanism of spirillar movement of bacteria

D'Arcy Thompson (p. 291) said that a "mechanism" was "whatsoever leads in the degradation of energy to its manifestation in some form of work". The gyratory undulatory movement of bacteria is a fact, and it is the bacterial body and not the flagellar apparatus which originates the movement. The energy then must come from the protoplasm or cytoplasm and must act on the cytoplasm and cell wall, producing spiral changes in shape. Cytoplasm according to D'Arcy Thompson (p. 347) is neither true fluid nor true solid; it has a micellar structure which gives it a certain rigidity, plasticity, tensile strength and ductility, and above all it has an ability to stream and flow. According to Seifriz (1936, p. 5) protoplasm usually appears to be quiet, but there are times when it flows rapidly. Protoplasmic streaming may be invisibly present in bacteria and may take place in spiral fashion, as in some higher plant cells, but it is doubtful whether it would be powerful or fast enough to set the bacterial cell in spiral motion.

Through the work of Preston (1939) and Astbury and Preston (1940) a good deal has become known of the structure of the cellulose wall of plant cells, especially algæ. Through methods of organic chemistry, X-ray analysis and polarisation optics they showed that such cell walls consist of many lamellæ, built of parallel fibrillæ which run in alternating spirals in the different layers. There is thus a spiral pattern in the wall of elongated plant cells. Cellulose is an aggregate of cellobiose units and these are built of long molecular chains which

are again grouped in micellæ. Protoplasm also consists of long chains of amino-acid residues. Preston therefore thinks that it may well be that the orientation of cellulose chains in the cell wall is brought about by a similar orientation of protein chains in the protoplasmic surface, because naturally the fundamental orientating spiral mechanism must lie in the cytoplasm. This means that in plant cells there would be a spiral configuration of protein molecules at the cytoplasm wall interface. If this holds good for bacteria, one can imagine that with such a spiral configuration of the protoplasm, wave-like spiral contractions of the protoplasmic membrane might easily come about which would then induce the cell wall to exhibit the spiral undulatory movement responsible for bacterial propulsion.

Classification of bacteria

Recent efforts at phylogenetic classification of bacteria in the wider sense still make use of morphological characteristics, although physiological characters also are introduced (Kluyver and van Niel, 1936 ; Stanier and van Niel, 1941). If, on the evidence here presented, bacteria which are usually described as rods are in future to be regarded as spirilla, and at the same time flagella are no longer regarded as motile organs but as somewhat accidental passive appendages, revisions will be necessary. Such revisions might clarify many issues. One example may suffice here. Starkey (1938) found spore-formation in *Vibrio desulfuricans* and expressed surprise, because vibrios and spirals are not supposed to do this. If however " bacteria " become, in effect, spirals, Starkey's finding may have a different significance.

SUMMARY

In solutions of gelatin or gum, motile bacteria move more slowly than in broth. The gelatin or gum is precipitated on the bacteria and their appendages, covering them with a sheath which interferes with motility and produces artefacts. In solutions of methylcellulose such artefacts can be avoided and the bacteria are slowed down by the viscosity of the medium. This affords a good opportunity for studying the nature of their motility. It is then seen that they exhibit a gyratory undulating movement, like other aquatic creatures.

That motile bacteria always exhibit a gyratory undulating movement was confirmed by making a slow motion cinemicrographic film of fast-swimming bacteria in broth, and also by examining the same bacteria at lower temperatures, which reduced their speed.

This spirillar motion of bacteria is sufficient to propel them and there is no need to invoke special motor organs like flagella. There is no evidence to show that the flagella-like appendages of bacteria act as motile organs—in fact all the evidence when critically examined points the other way.

Everything that has so far come to light as regards bacterial

flagella, whether by staining methods or electron microscopy, has been made visible by means of sunlight dark-ground microscopy, which must be regarded as providing better evidence,-since it deals with live bacteria in their natural state. This method has revealed that motile bacteria when swimming fast usually show a long tail, which is seen to be a spiral when speed diminishes. It can untwist into two " flagella " and this untwisting is reversible. Complete untwisting into a number of very thin wavy threads can also take place and this change is irreversible. It depends on the phase of untwisting in which a bacterium is caught by staining processes whether the result will be a bacterium with just one or two flagella or a bacterium surrounded by a fleece of wavy threads. The final picture is often complicated by the displacement of " flagella " and wavy threads during drying. Electron photomicrographs often show a number of the thin wavy threads lying about.

Analysis of the structure of bacteria excludes the possibility that tails, " flagella " or the thin wavy threads are live organs, or that they are in direct communication with the living parts of the cell. There is no evidence from either electron pictures or stained preparations that it is otherwise.

Not only does the visible gyratory undulating movement of motile bacteria satisfy all requirements for locomotion, but it is possible for bacteria grown under special conditions to swim in this fashion without showing tails or other supposed motor organs.

The shape of a motile bacterium during activity is therefore that of a helix or " spiral " and this must be regarded as their natural and normal shape. They should not be described as rods.

The motive force of bacteria resides in the cytoplasm and acts by means of wave-like spiral contractions which, through the cytoplasmic membrane, are communicated to the cell wall.

The slime layer which covers the cell wall is through these activities twisted into a tail which can split into two " flagella " or peel off into a number of thin wavy threads. This passive behaviour of the slime layer explains the various appearances of flagella-like structures of motile bacteria.

There is thus a vast difference between the flagella of protozoa, which are true motor organs, and the flagella-like appendages of motile bacteria, which are the result of motility.

The chemical nature of the slime layer and resulting appendages determines a kind of immunological specificity. Its physical nature and other factors are responsible for the number and position of the flagella-like appendages which become visible under various conditions. It is only, therefore, with great reservation that these structures can be used as differential diagnostic features. In any case, if, on the evidence here presented, most of the Eubacteriales are no longer to be regarded as rods but as spiral-shaped microbes, a good deal of revision of bacterial classification will become necessary.

I should like to express my indebtedness to Professor Robert S. Breed for suggesting and supplying methylcellulose, to Miss Rhona Brown for several drawings, to Miss Janet Todd for drawing and printing many illustrations, to Mr Leon Levson for expert photographic help and advice, and generally to the book *On growth and form* by Professor d'Arcy Thompson. Part of the apparatus used for these investigations was acquired through a grant from the Research Grant Board of the Union of South Africa.

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THE PREPARATION AND USE OF A SIMPLE CULTURE MEDIUM FOR LEPTOSPIRÆ

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PATHOGENIC leptospiræ are more fastidious in their cultural require-ments than leptospiræ of the *biflexa* type but they will grow in comparatively simple solutions ; 10 per cent. rabbit serum in water (Uhlenhuth, 1917) is adequate. Such media, however, frequently fail to produce cultures sufficiently luxuriant for use in serological tests. More satisfactory media such as the modifications of Vervoort's medium introduced by Schüffner (Davidson *et al.*, 1934) and by Korthof (1929) suffer from the disadvantage of phosphatic precipitation during sterilisation and are inconvenient to prepare in small quantities. The present medium was evolved in an endeavour to overcome these disadvantages. Following up the exhaustive work of Ono (1938), a large number of inorganic salts and carbohydrates were tested for their effect on leptospiral cultivation and the substances eventually selected were found to be helpful. The advantage of adequate buffering was fully confirmed but no buffer substance was found better than Sörensen's phosphate mixture. All attempts to replace serum by simpler or more uniform substances failed. It is hoped to record these results in detail at a later date, but since the medium below has already been referred to in published work (Buckland and Stuart, 1945) it seems desirable to give details of its composition.

MEDIUM

Concentrated solutions of most of the substances used can be kept in stock for an indefinite period and, from these, 200 ml. quantities of the final medium are prepared as required. From this, small volumes can be distributed into culture tubes. The bulk medium remains satisfactory for about a month but later becomes gradually more acid, probably due to CO_2 absorption. If the medium is likely to be used only occasionally it can be dispensed in 1-oz. screw-cap bottles and will keep unimpaired for at least a year if the bottles are full.

With a sterile 10-ml. pipette the volumes given in table I are measured into a very carefully cleaned screw-cap bottle, the pipette being rinsed with boiling water between each operation. If the ingredients are pipetted in the order given, bacterial contamination does not appear to become established in the basic solutions. The mixture is steamed for 30 minutes to drive off CO_2, as also, at the same time, are 20 ml. of Sörensen's buffer solution pH 7·6 prepared

as follows :—Na$_2$HPO$_4$.2H$_2$O (11·876 g. per l.) 17·6 ml. ; KH$_2$PO$_4$ (9·078 g. per l.) 2·4 ml. : 16 ml. of the buffer mixture are then added to the rest of the medium and sterilisation completed by steaming for one hour.

TABLE I

Composition of basic medium, less phosphate

Material	Concentration	Volume (ml.)	Final concentration
Asparagin (dextro-rotary) . .	M/10	2	M/1000
NH$_4$Cl	M/10	10	M/200
MgCl$_2$	M/10	4	M/500
NaCl	M/10	66	M/30
Glycerin A.R.	...	1	0·5 per cent.
Phenol red in Aq. dest. . .	0·02 per cent.	10	0·001 „
Aq. dest.	...	91	...

For use, 2-3 ml. of the medium are pipetted aseptically into carefully washed tubes or screw-cap bottles, 5-10 per cent. of sterile rabbit serum is added, and the completed tubes or bottles are placed in a water-bath at 60° C. for one hour.

PRECAUTIONS

Attention to certain minor details is very important if satisfactory cultures are to be obtained.

Preparation of glass-ware. All glass-ware must be scrupulously clean, since even a trace of soap is lethal to leptospira in culture. Culture tubes are washed and rinsed in the usual way and then steeped in phosphate buffer solution at *p*H 7·6 for twenty-four hours before being finally rinsed and sterilised. Good quality cotton-wool is essential if plugged tubes are used, and plugs must not be flamed during use.

Selection of serum. Filtered rabbit serum is best and the *p*H should be checked after filtration in case of a swing to the alkaline side. Occasional rabbit sera are useless and these can be eliminated only by trial. The suggestion of Smith (1937) that such sera contain leptospiral antibodies due to natural rabbit infection applies in only a very few instances—one rabbit in over two hundred tested. Pooled guinea-pig serum, being more uniform, may be used, but is never quite so satisfactory. Other sera such as ox, sheep and human are invariably inferior.

Inoculum. This should be heavy—about 0·5 ml. of a well grown culture into 3 ml. of medium. If cultures used for routine serological work are sub-cultured weekly excellent growths can confidently be expected.

Incubation. If incubated at 30° C. cultures are usually ready for use after four days. Growth at lower temperatures—even room temperature—is quite satisfactory but somewhat slower ; incubation at 37° C., while it may accelerate growth in well established strains, is apt to cause rapid degeneration.

Weak or feebly viable strains. These strains are much more susceptible to variations in the quality of the serum used ; with a very good serum little trouble may be experienced, but with others one may get poor or feeble growths which have to be built up for several weeks before they become suitable test antigens. When cultures are very feeble they may be revived more readily by adding fresh medium to the original culture tubes than by subcultivating.

Contaminants. Frank contamination is easily detected by the development of gross turbidity. Slowly growing contaminants are much more troublesome, because for several subcultures they may cause little alteration in the macro-

scopic or microscopic appearance of the leptospiral culture, and when their presence is at last recognised it may be too late to retrieve the strain from older pure cultures. In my experience bacteria which interfere with the viability of leptospira invariably cause increasing acidity, easily detected by the incorporated indicator. Such cultures can be purified by the method of rapid guinea pig "filtration" described by Schuffner (1040) and detailed by Buckland and Stuart. A simpler method, often satisfactory, is based on a technique of Stavitsky (1045), who used sulphanilamide in a concentration of 400 mg. to 100 ml. of his medium when attempting to cultivate leptospira from contaminated biological material. On the evidence presented by Jacoby (1945) sulphaguanidine was tried and found much better than either sulphanilamide or sulphadiazine. A saturated solution of this drug is prepared in the basic medium and various concentrations are used to allow for the variable resistance of different strains of leptospira. A mixture of 4 parts of the saturated solution with 6 parts of the ordinary basic medium is a satisfactory average concentration. If active leptospiræ are still numerous in the contaminated culture this method is usually adequate. Slowly growing contaminants which do not alter the *p*H of the medium cause little trouble; one strain of *L. ictero-hæmorrhagiæ*, for instance, was maintained for more than two years in association with an accidentally introduced strain of *Bact. alkaligenes* which ultimately died out. The leptospiral strain is still healthy three years later. Such an occurrence, however, is unusual.

COMPARATIVE OBSERVATIONS ON VARIOUS MEDIA

Growth of strains of leptospiræ adapted to artificial culture

The media chosen for comparison were those of Schuffner (1940), Korthof (1929), Brown (1935), Vervoort (described by Hindle, 1931) and Uhlenhuth (1917). They were distributed in equal volumes in sterile test tubes. Filtered rabbit serum was added. The cultural quality of any medium depends largely on the particular rabbit serum employed, and since a very considerable variation exists between sera a large number of sera were used. Inhibitory sera were eliminated by preliminary tests. Most of the tests were carried out with strains of *L. icterohæmorrhagiæ*, of which 25 different strains were used, a few with *L. grippo typhosa* (strain Moscow V). *L. canicola* strains were found too adaptable to give satisfactory comparative results. Inocula were grown in a variety of media to avoid adaptation to any particular medium, but to make the test more severe they were much smaller than those used in routine work, one drop per ml. being the average. Parallel tubes of the above-mentioned media and of the newly described medium (called "A" for convenience) were inoculated with equal volumes of the same leptospiral cultures and incubated at 27 30° C for seven days.

Tests were carried out during routine work at intervals over several years and the results in the separate sets of tests were often very different. The collected results are shown in table II, in which "standard density" means a density considered suitable for serological work.

It must be noted that all the media tested are fairly satisfactory for routine use and that Schuffner's, Korthof's and Brown's will regularly produce excellent cultures if inoculated with adequate volumes of active leptospiræ. Table II does not show the number of occasions on which these media produced cultures of standard density more luxuriant than medium "A", and the severity of the

tests undoubtedly accentuated certain minor differences, with an unexpected advantage to the latter medium.

TABLE II

Results of comparative cultures on medium A and other media

Strains of leptospira	Different sera used	No. of cultures	Number of cultures showing standard density (S.D.) in				Second medium
			medium A		second medium		
			S.D.	Percentage	S.D.	Percentage	
13	29	65	47	72	34	52	Schüffner
7	3	25	16	64	9	36	Korthof
25	4	36	18	50	11	30	Brown *
7	3	12	8	67	2	17	Vervoort
4	2	14	9	64	0	...	Uhlenhuth

* This test was particularly severe, since 10 of the strains used were old and feebly viable; 8 did not grow in Brown's medium and 2 did not grow in " A ".

ISOLATION OF LEPTOSPIRÆ FROM INFECTED ANIMALS

Most of the comparative tests were made by distributing one or two drops of infected guinea-pig's blood into parallel tubes of culture media as before. A few isolations were accomplished by direct culture of small pieces of rats' kidneys. Cultures were examined at intervals up to three weeks.

Seven strains of leptospiræ were obtained from cultures in 23 parallel tubes of Schüffner and " A ". Although parallel cultures were rarely alike, yet the final results were exactly equal, each medium being superior to the other on nine occasions and equal on five. Similar results were obtained in a smaller comparative series with Korthof's medium, which was superior to " A " on two occasions, inferior in other two and equal twice.

Results with the same strain and the same culture medium can often be dramatically different on different occasions and probably depend on the power of adaptation of individual organisms to artificial conditions. This is well exemplified by the instance shown in table III, where similar volumes of infected blood obtained by heart puncture on different days were introduced into parallel tubes of Schüffner and " A " media.

Maintenance of stock cultures of leptospiræ

One of the main difficulties in leptospiral work is the maintenance of strains without frequent subculture. For several years I used Fletcher's (1927-28) medium tubed in 5 ml. volumes, and found that leptospiræ kept at room temperature generally remained viable for one month and often for two months, while infrequently cultures lived for longer than three months. The duration of viability depended largely on the luxuriance of the cultures. Rich cultures tended to

die out more rapidly than poor cultures and the luxuriance of the growth in Fletcher's medium militated against prolonged life The

TABLE III

Successive blood cultures from a guinea pig inoculated with rat kidneys
(Same batch of medium used in both instances)

Guinea pig heart puncture	Incubation (days)	Growth of leptospira in	
		Schüffner	A
21/10/40	3	—	—
	7	—	sc
	8	sc	+
	11	sc	+ + + +
23/10/40	1	sc	sc
	5	sc	sc
	8	sc	sc
	12	+ + +	—

Sc = scanty (less than 10 leptospira in a low power field)
+ to + + + + = gradations in culture up to maximum luxuriance

' A " medium with the addition of 0 1 to 0 2 per cent of agar suffered from the same defect Out of 125 cultures only 118 survived for five weeks Even when cultures in Fletcher's medium or in this variation of the ' A " medium appeared viable microscopically, it was frequently necessary to subcultivate to strictly fluid media (Schüffner, Korthof, ' A ", etc) to revive the organisms Fluid media alone, however, were inferior to the semi solid media mentioned above, and the " A " medium was somewhat less satisfactory than Schüffner's or Korthof's Mino (1941) published a special method for preserving cultures in which roughly 8 per cent of fresh guinea pig's blood was thoroughly mixed with Korthof's basic medium and then pipetted into narrow tubes previously treated with liquid paraffin In a test of this medium 13 strains of leptospira were grown in culture tubes prepared from the blood of one guinea pig and 11 strains in similar tubes containing blood from another guinea pig Only four strains of the first batch and five of the second survived for one month Nevertheless two strains, one in each batch, were still alive and could be subcultivated more than seven months later One of these was successfully sub cultivated after a total period of 13 months 19 days I have not investigated the matter further, but this finding suggests that Mino's method might be more successful if his technique were more fully described than in his original paper Mino's use of guinea pig blood suggests that guinea pig serum might be better than rabbit serum, since it rarely produced such luxuriant growths Accordingly I tested 20 strains of *L icterohæmorrhagiæ* in 3 ml volumes of my " A " medium prepared from several different batches of pooled

guinea-pig serum. Records kept over several months gave the results shown in table IV.

<div align="center">TABLE IV</div>

Viability of leptospiræ in " A " medium with guinea-pig serum

No. of cultures	Number of cultures viable after (days)			
	30-40	40-60	60-75	75-90
59	59	...	...	...
75	75	74	74	...
43	43	43	42	38

These strains of *L. icterohæmorrhagiæ* were maintained for over a year in medium " A " prepared with guinea-pig serum and were regularly subcultivated with success after 3-6 weeks at room temperature. For the purpose of maintaining leptospiral strains guinea-pig serum has proved more suitable than rabbit serum, and pooled guinea-pig serum is less variable than individual rabbit sera but each batch should be tested before use.

DISCUSSION

The " A " medium described above has been in use for six years, but the results given in the tables are distinctly surprising to me. The comparative tests were carried out intermittently during this period and the results were collected together immediately before this paper was written. Early results had suggested that my medium was as good as, but not better than, any of the others in common use. Even yet, I think that Schüffner's and Korthof's media have a slight advantage from a general cultural point of view, because they appear to be less sensitive to the quality of the serum employed. In my opinion, however, the other advantages of the " A " medium outweigh this minor disadvantage. It is simple ; 200 ml. of basic medium can be prepared in 10 minutes, though sterilisation requires an hour and a half longer. Phosphatic precipitation does not occur. The incorporation of an indicator has been very helpful on numerous occasions in drawing attention to some deviation in reaction likely to be harmful to culture or to serological test. The use of glycerin, because of its experimentally discovered growth-promoting properties, has the unexpected advantage of delaying for several minutes the drying of loopfuls of culture examined without cover glass as in serological tests, thus allowing more drops to be examined at one time and giving a longer period for inspection. Preliminary heating of the medium at 60° C. has some sterilising value and does not interfere with its cultural properties ; a few sera, however, become

slightly cloudy at this temperature and in such cases heating should be avoided if a crisp dark-ground picture is desired.

The medium has proved adequate for the isolation of leptospiræ from infected men and animals and the use of guinea-pig instead of rabbit serum has made it satisfactory for the maintenance of stock cultures, thus eliminating the duplication of media. In comparative tests it has proved at least the equal of any other commonly used medium and it has certain distinct advantages which have been indicated above. Nevertheless it shares with other media the disadvantage of being dependent very largely on the quality of a variable biological product—animal serum, and although it is commonly subjected to a higher temperature than is usual in the preparation of such media it cannot be heat-sterilised ; its sterility depends therefore on technical skill and good luck.

CONCLUSIONS

A new and simple culture medium for leptospira is described and compared with certain commonly used media. It is satisfactory for the isolation of leptospiræ, for their maintenance in stock cultures and for serological work in leptospiral infections.

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THE RELATIVE IMPORTANCE OF THE RENAL AND HEPATIC LESIONS IN EXPERIMENTAL LEPTOSPIROSIS ICTEROHÆMORRHAGICA

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(PLATES L AND LI)

In the course of a study of the pathogenesis of experimental Weil's disease, a strain of *Leptospira icterohæmorrhagiæ* has been maintained in a state of virulence by passage through guinea-pigs. Post-mortem examinations carried out as a routine on the animals dying of this infection revealed surprising variation in the extent of liver necrosis, a lesion to which earlier workers have attached first importance (Stokes and Ryle, 1916 ; Stokes, Ryle and Tytler, 1917 ; McNee, 1919-20 ; Buchanan, 1927). In spite of this, no alteration in the survival time of the guinea-pigs, which for this strain was stable at 8 days for an animal of 300-400 g. weight, was noticed, nor did there appear to be any difference in the intensity of the jaundice in those in which little or no hepatic parenchymatous necrosis was visible. These casual observations led to enquiries being undertaken into the cause of death in the experimental animal and attention was turned to the kidney, which has long been recognised to be damaged in leptospirosis. Indeed the clinicians of the latter part of the last century, doubtless influenced by the work of Bright (1836) and lacking elaborate diagnostic facilities, paid more attention to the renal manifestations of human Weil's disease than to the hepatic.

METHODS AND MATERIALS

Experimental animal

The experimental animal used throughout this investigation was the guinea-pig. Only animals weighing 300-400 g. were used, with equal numbers of the two sexes. The diet comprised an excess of the following articles :— moistened bran, alfalfa (Lucerne), and brassica leaves or Swedes. The animals were fed each day. This diet has been found to be adequate for the maintenance of perfect health in the guinea-pigs used by the Emergency Public Health Laboratory, Oxford, for routine diagnostic purposes.

Strain of Leptospira icterohæmorrhagiæ

The same strain of *L. icterohæmorrhagiæ* was used throughout. It was obtained from the kidneys of rats infesting an Italian prisoner-of-war encamp-

ment in which two serologically established cases of Weil's disease had occurred, both of them of the severe jaundiced type. The organism was maintained over the required period by continuous guinea-pig passage carried out in the following manner. Two guinea-pigs were maintained as a reservoir for the strain. As soon as possible after the death of an animal the kidneys were removed, cut up with scissors into a pulp and suspended in one-quarter strength Ringer's solution. One ml. of the resulting suspension was injected into two guinea-pigs intraperitoneally. The presence of leptospiræ was always confirmed by examination of the kidney pulp by dark ground illumination. After the first three guinea-pig passages the virulence of the strain became stable, killing a 300-400 g. guinea-pig in eight days. The lesions found at autopsy were characteristic, with extensive hæmorrhages and profound icterus.

Experimental methods

Twenty guinea-pigs in all were infected intraperitoneally with 1 ml. of the kidney suspension prepared as described above. Early on the eighth day after inoculation, when all the animals were *in extremis*, blood was taken by cardiac puncture and the blood urea estimated by the method of Archer and Robb (Harrison, 1939), the estimations being made colorimetrically against freshly prepared standards in a Leitz compensating colorimeter. At the same time an equivalent number of normal guinea-pigs had their blood urea estimated in the same way.

RESULTS

These are summarised in tables I and II. Histological examinations were carried out on the kidneys of all these animals and figs. 1

TABLE I

Blood urea estimations on 20 normal guinea-pigs

Animal no.	Blood urea (mg. per 100 ml.)	Animal no.	Blood urea (mg. per 100 ml.)
1	10·1	11	13·8
2	15·0	12	19·0
3	12·4	13	21·5
4	20·2	14	12·0
5	18·5	15	10·5
6	23·2	16	16·0
7	18·9	17	20·0
8	11·6	18	11·4
9	13·5	19	13·0
10	25·2	20	19·2
Average . 16·86		Average . 15·64	
Total average . . 16·25 mg. per 100 ml.			

and 2 show the lesion found in all those dying from leptospirosis icterohæmorrhagica. The lesion comprises a severe engorgement of the glomerulus, with tubular damage similar to that encountered in several diseases of differing ætiology—incompatible blood transfusion (Holman, 1939), traumatic anuria (Bywaters and Dible, 1942) and blackwater fever (Maegraith and Findlay, 1944).

LIVER AND KIDNEY IN SPIROCHÆTOSIS ICTEROHÆMORRHAGICA

FIG 1 —Kidney in experimental spirochætosis icterohæmorrhagica, showing intense engorgement of glomerulus and slight thickening of the epithelium of Bowman's capsule Mallory's trichrome stain   ×440

FIG 2 —Kidney in experimental spirochætosis icterohæmorrhagica, showing necrosis of tubular epithelium, with lumina containing cell debris Mallory's trichrome stain ×440
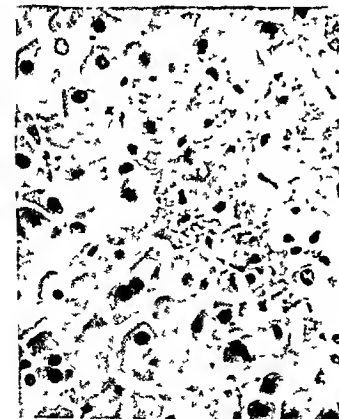
FIG 3 —Liver Control series, showing cytoplasmic shrinkage and nuclear pyknosis Area of necrosis with complete disappearance of nuclei in upper right quadrant Hæmatoxylin and eosin   ×440
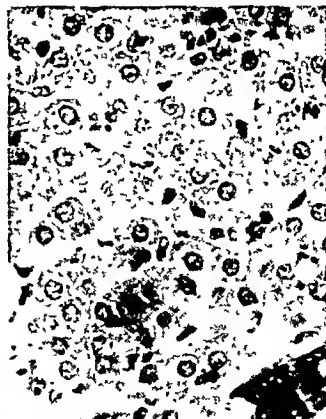
FIG 4 —Liver Methionine treated series, contrast with fig 3 Hæmatoxylin and eosin ×440

In view of the apparent uniformity in the degree of renal damage occurring in this series it was decided to attempt to vary still further the liver damage found to occur naturally. Since the original observations of Davis and Whipple (1919) and of Miller and Whipple (1940) that a diet rich in protein protected the liver from necrosis in chloroform poisoning, a considerable body of literature has grown up on this subject. Himsworth and Glynn (1939-42) showed that a high protein diet was more efficacious in the prevention of liver damage in experimental T.N.T. poisoning than a carbohydrate diet, while the administration of fat was detrimental to the liver. Subsequent investigators have demonstrated that the sulphur-containing amino acids, particularly methionine, were the essential protective factors (György and Goldblatt, 1937 ; Daft, Sebrell and Lillie, 1941). Glynn and Himsworth (1944) demonstrated in rats that methionine specifically inhibited hepatic necrosis caused by protein-deficient diets and suggested the differentiation of trophopathic from toxipathic liver necrosis, the former resulting from a dietary deficiency which methionine specifically prevented, the latter being due to the direct action on the liver parenchyma of an exogenous toxic body in the prevention of which methionine was less effective.

The experience of these workers led me to try the effect of methionine upon guinea-pigs infected with *L. icterohæmorrhagiæ* receiving an adequate diet.

TABLE II

Blood urea estimations on guinea-pigs 8 days after inoculation with L. icterohæmorrhagiæ

Guinea-pig no.	Blood urea (mg. per 100 ml)	Guinea-pig no	Blood urea (mg. per 100 ml.)
1	224	11	260
2	232	12	221
3	220	13	250
4	213	14	266
5	218	15	241
6	227	16	214
7	216	17	250
8	229	18	220
9	224	19	219
10	239	20	248
Average . 223·3		Average . 238·9	
Total average . . 231·3 mg. per 100 ml.			

receiving an adequate diet. Forty pairs of animals were used for this investigation (table III), 20 of which were treated with 1 mg. per kilo body-weight of methionine per day, administered subcutaneously, while the remaining 20 pairs were left as untreated controls. All 80 animals were inoculated intraperitoneally one after the other with

1 ml. of a suspension of the four kidneys of two guinea-pigs which had died of the disease : this inoculum contained from 5 to 8 living motile leptospiræ per 1/12″ dark ground field. Thus it was ensured that each animal received approxinately the same number of infecting organisms. Care was taken to see that both the treated and the

<p align="center">TABLE III</p>

<p align="center">*Incidence of liver necrosis in guinea-pigs infected
with L.* icterohæmorrhagiæ</p>

	Liver necrosis present	No liver necrosis	Total
1st series			
Treated	2	38	40
Controls	26	14	40
	28	52	80
2nd series			
Treated	1	11	12
Controls	7	5	12
	8	16	24

control series had the same diet and that each group contained an equal number of each sex. All the animals died on the eighth day after inoculation and at autopsy careful note was made of the presence or absence of liver necrosis and specimens of this organ and of the kidneys were taken for histological examination.

In view of the observed variation in the incidence of hepatic necrosis in the experimental disease the results pertaining to this point have been analysed statistically by Mr P. H. Leslie of the Bureau of Animal Population, Oxford University. This investigation was repeated over 12 pairs of guinea-pigs not simultaneously inoculated, the results of which have been similarly analysed.

Applying Yates's correction for continuity, we have for the first series of experiments $\chi^2 = 29 \cdot 07$, P = $< \cdot 001$, and for the second series $\chi^2 = 4 \cdot 69$, P $< \cdot 05$, $> \cdot 02$. In both series, therefore, the difference between the treated and control animals is significant. It is evident that there is little difference between the percentage of treated animals showing necrotic lesions in the two series, and similarly for the controls. The average over both series is 3 : 52 or $5 \cdot 77 \pm 3 \cdot 13$ per cent. of treated animals with necrotic lesions and 33 : 52 or $63 \cdot 46 \pm 6 \cdot 68$ per cent. of controls.

Blood urea estimations carried out on both control and treated

animals in every case amply confirmed the results of the first experiment described, the average figures being as follows :—

Controls	.	.	.	.	212 mg. per 100 ml.
Treated	.	.	.	.	216 ,, ,, ,, ,,

It will therefore be seen that although methionine significantly reduces the liver necrosis occurring in leptospirosis icterohæmorrhagica in the guinea-pig, there is no variation in the survival time after infection nor is the degree of renal failure in any way influenced.

Certain other features of interest were noticed in these investigations. Where no actual liver necrosis was to be seen in the control animals the whole organ was extremely friable, whereas in the treated animals it was much firmer to the touch. This difference in consistency may be accounted for by the marked shrinkage and wide separation of the parenchymal cells observed in the control series (fig. 3), but not present to the same degree in the liver of animals which had received the additional methionine. Here, in spite of engorged sinuses, little cellular damage seems to have occurred (fig. 4). There was no difference in the intensity of the jaundice on naked eye comparison of the sera depending on the presence or absence of liver necrosis. Where necrosis was extensive and confluent, as in four of the control series, it was found that the hæmorrhages in the lungs, usually punctate or small (fig. 5), were much more extensive, often involving an entire lobe (fig. 6). Similarly, large hæmorrhagic bullæ were found in the subserosa of the intestine. These hæmorrhagic manifestations were never seen in the groups treated with methionine. A possible explanation is that where liver necrosis is severe the clotting time is prolonged, thus allowing extravasation of blood to occur more readily, especially in the moving viscera.

Discussion

From the experiments described it appears clear that the liver necrosis occurring in experimental Weil's disease in the guinea-pig is a variable feature and that a statistically significant reduction in its incidence can be effected by the administration of methionine over and above that contained in an adequate diet. Irrespective of the liver necrosis, the renal lesion seems to be uniformly severe and death appeared to be due invariably to renal failure.

The relatively greater importance and constancy of the renal lesion in the complex syndrome of leptospirosis icterohæmorrhagica tended to be overlooked in the past owing to attention being focussed on the jaundice and the role played by the liver in its origin. Stokes, Ryle and Tytler considered that it was due to obstruction following pericholangitis but Beitzke (1916) found no evidence of obstruction. I have investigated the mechanism of icterogenesis experimentally and hope to include the results of this work in a later publication.

Martin and Pettit (1919) did not attempt to explain the ictero-genesis but significantly drew attention to the greatly increased blood destruction taking place in the spleen. Buchanan (1927) in his monograph on leptospiral jaundice, examined much human and guinea-pig material from which he obtained a wealth of hæmatological data and drew attention to the polymorphonuclear leucocytosis accompanying Weil's disease, thus helping to distinguish it from epidemic hepatitis, which is usually associated with a leucopenia. This worker, however, gave the liver lesions first place in his description of the pathological changes and omitted to undertake any renal function tests.

While it is unwise to draw analogies too freely from the experimental disease, it is of interest to note that, as the agglutination test is being more extensively employed in Weil's disease, attention is being directed away from the jaundice and liver lesions. In an extensive survey of the leptospiroses in the Netherlands and the Dutch East Indies, Walch-Sorgdrager (1939) showed that 40 per cent. of serologically established cases of Weil's disease failed to develop signs of hepatic insufficiency, and Gardner (1943) has stressed the fact that absence of jaundice does not exclude the possibility of this disease. With the extended use of serological methods and of muscle biopsy (Sheldon, 1945) in diagnosis, the proportion of anicteric cases of Weil's disease may well prove to be higher than has been recognised hitherto. Maclagan (1944), working on the colloidal gold reaction of the serum, finds that in diseases invariably involving the liver, such as cirrhosis and infective hepatitis, the reaction is positive in over 90 per cent. of cases, whereas in sera agglutinating *L. icterohœmorrhagiæ* the result is very variable.

Recent workers on the human infection have attached greater importance to the renal aspect of the syndrome. Borgen and Thjotta (1941) describe severe anicteric cases with blood urea values up to and greater than 300 mg. per 100 c.c. Borgen (1941) states that he finds leptospiræ more consistently in kidney than in liver tissue. Senekjie (1944), reviewing the incidence of the human disease in Lousiana, states that in all thirty of his cases evidence of renal damage was found. In a total of 39 cases occurring among troops, Bulmer (1945) records 3 fatal cases, two of which were said to have succumbed to uræmia. All these observations on the human disease are in conformity with the results obtained in the guinea-pig. It has been shown here that severe renal damage with greatly increased blood urea was a constant feature of animals dying of the disease, irrespective of the severity of the liver lesions in the controls and its mitigation by the administration of additional methionine.

The significance of the effect produced by methionine cannot at this stage be interpreted. It has not so far been shown that guinea-pigs if deprived of this amino acid incur liver necrosis, as is apparently the case with rats (Himsworth and Glynn, 1944-45).

LUNGS IN SPIROCHÆTOSIS ICTEROHÆMORRHAGICA
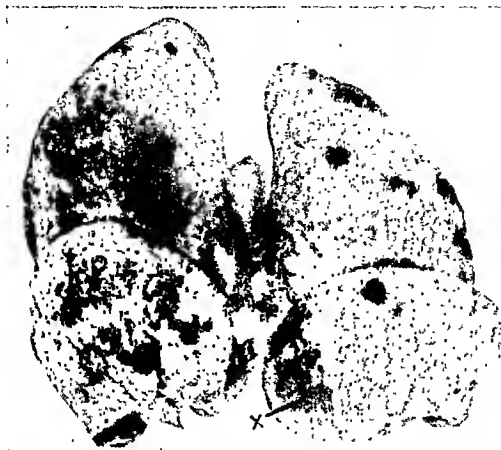
FIG. 5.—Lungs, showing the usual appearance in the experimental disease in the guinea-pig, with small areas of hæmorrhage. The area × is not hæmorrhagic but is due to a lighting effect. × 1·1.

FIG. 6.—Lungs in spirochætosis icterohæmorrhagica, showing extensive confluent hæmorrhages coincident with severe liver necrosis. × 1·5.

Attention has already been drawn to the histological appearance of the renal lesions (figs. 1 and 2). The similarity to that occurring in other pathological conditions of widely differing ætiology has been shown by Maegraith, Havard and Parsons (1945). These authors have suggested that a state of renal anoxia is responsible for the lesion in this group of conditions, which they have called the "syndrome of renal anoxia". We have obtained evidence in support of this viewpoint, which will form the subject of a subsequent communication.

SUMMARY

1. Necrosis of the liver is an inconstant feature of experimental leptospirosis icterohæmorrhagica in the guinea-pig.

2. The administration of additional methionine produces a statistically significant reduction in the incidence of liver necrosis.

3. The degree of liver damage present fails to influence the intensity of the jaundice or the time of survival of the animal after inoculation.

4. A renal lesion comprising severe congestion of the glomeruli and destruction of the tubular epithelium is a constant feature of the disease and death appears to be due to renal failure irrespective of coexistent liver damage.

Addendum

Since this paper was submitted for publication Hutchinson and his colleagues (1946) have described an outbreak of Weil's disease in the British Army in Italy. These authors remark upon the absence of hepatic damage found at autopsy in their 6 fatal cases.

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THE BACTERICIDAL POWER OF THE BLOOD FOR THE INFECTING ORGANISM IN BACTERIÆMIA

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The measurement of the bactericidal power of human blood presented in this paper was undertaken as a test of the validity of the pathogen-selective culture method, which was devised by Solis-Cohen (1927; see also Wright, 1915) for the selection from mixed cultures of organisms suitable for autogenous vaccines, on the assumption that the organisms which resist killing by the patient's fresh whole coagulable blood are ætiologically important. The material to be examined is incubated for 24 hours with 3-5 c.c. of the blood; after 24 hours the clot is removed and the organisms growing from the remaining blood are considered to be responsible for the infection.

Boerner and Solis-Cohen (1933) studied 404 pathogen-selective cultures from 150 persons. The cultures were taken mostly from the nares, naso-pharynx and tonsils, and in a few cases from the sputum, teeth, urine and fæces, an anal fissure and a carcinomatous ulcer. These sites are exposed and heavily infected with commensals and secondary invaders, and the chances of isolating any " etiologically important " organism from them are remote. Solis-Cohen (1936) attributed the contradictory results of experiments with the method previously employed by various workers to the different species of bacteria, different species of animals and different methods and types of blood used.

The validity of the assumption that the blood will kill all bacteria except those responsible for the infection can be tested by measuring the bactericidal power of an infected patient's blood against the infecting strain in diseases where there is no doubt of the infective agent of such diseases. General infections accompanied by bacteriæmia are clearly suitable. This communication reports the results of 22 tests made on 21 patients with bacteriæmia as soon as a bacteriæmia strain had been isolated. The series contained no patients receiving chemotherapeutic drugs ; the present tendency to administer chemotherapeutic drugs immediately after a blood culture is taken is responsible for the small number of patients in the series.

Method

The original blood culture was plated and a single colony incubated for 24 hours in broth. About 20 c.c. of blood were drawn from the patient and 15 c.c. immediately defibrinated with sterile glass beads. Serum from the remainder was used for an agglutination test with the bacteriæmia organism. The defibrinated blood was filtered through sterile muslin, thoroughly mixed and distributed in 0·4 c.c. quantities in eighteen 3-in. × ½-in. tubes. One 0·02 c.c. drop of 1·25 per cent. sodium polyanethol sulphonate (Liquoid) in saline was then added to half the tubes to inhibit the bactericidal power. One c.c. of the defibrinated blood was also used in making an agar plate to determine the number of bacteria originally present in the circulating blood of the patient at the time of withdrawal.

Measurements were made by means of standard pipettes delivering 50 (0·02 c.c.) drops to the c.c. Two series of 9 tubes, one containing blood alone, the other sulphonated blood, were seeded with 0·1 c.c. of 8 tenfold dilutions of culture, one dilution to each tube, the last tube in each series being left as an uninoculated control. At the same time samples were taken from the culture dilutions for a surface viable count (Miles and Misra, 1938), thus ensuring a count of the inoculum at the time of mixing with the blood. The blood tubes were sampled immediately after inoculation and again after 2 hours at 37° C., with shaking every 10 minutes. The sampling of the tubes containing sulphonate confirmed the viable count made on the culture dilutions.

The samples were seeded by letting fall four 0·02 c.c. drops of each blood-bacterium mixture on to numbered sectors on each of, four well dried plates of an appropriate medium and the survival rate of the organisms estimated by the method of Miles and Misra. The colonies developing were counted after 24 and 48 hours at 37° C., and after 24 hours' incubation a loopful of each blood-bacterium mixture was streaked on to plates.

Twenty-two tests were made on the blood of 21 bacteriæmic patients. In twenty instances the strains tested were isolated from the blood; in one case the strain from a recurrent ulcer on the patient's leg was used (test 19, table II). The organisms are listed in table II.

The result of test 1 is detailed in table I as an example of the method used. The ætiological importance of the organism (*Bact. coli*) was clear. It was

TABLE I

Showing detailed results of test 1 in table II

Inoculum in 0·02 c.c. of blood-bacterium mixture	Mean counts of four 0·02 c.c. drops of blood-bacterium mixture					
	Without sulphonate, sampled at			With sulphonate, sampled at		
	<5 mins.	2 hours	24 hours	<5 mins.	2 hours	24 hours
49,200	c	u	C	C	C	C
4920	u	20	C	C	C	C
492	6	1·5	C	c	c	C
49	1·7	1	0	49·0	u	C
4·9	0·3	0	0	4·2	12	C
ca 0·5	0	0	0	0·7	0·7	C
0	0	0	0	0	0	0

C, c = grades of confluent growth.
u = mainly discrete colonies, but uncountable.

isolated from the blood and urine in large numbers, and both the blood and urinary strains, which were biochemically identical, were agglutinated to the

same titre (1 : 80) by the patient's serum but were not agglutinated by pooled normal human sera.

For convenience, all counts are recorded as the bacterial content of a 0·02 c.c. volume of fluid. The viable count of the 24-hour broth culture, estimated from a 6-drop count on 3 plates, was $2·46 \times 10^8$ per 0·02 c.c. The concentration in the corresponding blood-bacterium mixture was therefore $2·46 \times 10^8/5 = 4·9 \times 10^5$ per 0·02 c.c. The blood as drawn contained 3 organisms per c.c., so that 0·06 was present in the standard 0·02 c.c. sample of blood in the test, in addition to the added bacteria. A test on the defibrinated blood alone showed that these " native " organisms were rapidly killed by the blood.

The counts (table I) in the sulphonated mixtures show a progressive increase in the numbers of bacteria. Incidentally the initial numbers of bacteria in the mixtures as estimated from a viable count of the culture used and as measured directly, immediately after mixing, are in substantial agreement. In the non-sulphonated tubes killing is marked even in mixtures that had been in the tube only a few minutes, indicating that, as Miles and Misra pointed out, killing of the bacteria continues in the blood drop after it has been seeded on to the agar plate. Killing is most marked after 2 hrs. : twenty organisms survive out of 4920 and 49 are completely killed. The survivors clearly cannot be held in check for 24 hours, when most of the mixtures containing living bacteria at 2 hours are overgrown.

In table II only the counts in the non-sulphonated mixtures are recorded ; in all the sulphonated controls the expected progressive growth of bacteria occurred, showing that in all cases the sulphonate destroyed whatever bactericidal power the blood possessed.

Results

It appears from table II that susceptibility or resistance to the bactericidal power of the blood is determined by the nature of the bacterial species and not by the fact that the organism tested is responsible for the infection. Thus in seven undulant fever infections the blood of the hosts had in no case any bactericidal action upon the infecting organism : on the other hand in 5 instances (2 *Bact. coli*, 1 *Bact. typhosum* " O ", 1 *Staph. aureus* and 1 *viridans* strepto-coccus) the blood exhibited marked killing properties. It is of interest that a sample of healthy human blood tested in a similar manner had no bactericidal power against the strain *Br. abortus* 2.

The bacteria tested can be divided into 3 groups :

(a) Those which were fully susceptible to the bactericidal power of the blood : 2 *Bact. coli* (tests 1 and 2) ; 1 *Staph. aureus* (test 3) ; 1 *Bact. typhosum* " O " (test 4) ; 2 *viridans* streptococci (immediately acted upon, but survivors multiplied in 2 hours : tests 5 and 6).

(b) Those which were slightly susceptible : 1 *Bact. paratyphosum C* (test 20) ; 2 *Staph. albus* (tests 21 and 22).

(c) Those which were resistant : 7 *Brucella abortus* (tests 8-14) ; 1 *Bact. typhosum* (test 15) ; 1 *Bact. typhosum* " O " (test 16) ; 1 *Bact. paratyphosum A* (test 17) ; 1 *Bact. coli anaerogenes* (test 18) ; 1 *Streptococcus pyogenes* type III (test 19).

There is some evidence that the susceptibility to the bactericidal power of the blood differs from strain to strain in the same species,

Table II—*The bactericidal power of the patients' blood for the infecting strain in 21 cases of bacteriæmia*

Test no.	Organism tested and its source	Duration of illness (days) before isolation of test-strain	Mean counts in 0·02 c.c. volumes of blood-bacterium mixture				Reciprocal of homologous agglutinin titre
			Initial	>5 mins.	2 hours	24 hours	
1	Bact. coli 1. (Pyelitis, bacteriæmia)	4 pyrexia	4920	n	20	0	<80
			492	6	2	0	
			49	2	1	0	
			5	0	0	0	
2	Bact. coli 2. (Pyrexia, bacteriæmia and bacilluria)	20	2210	77·5	26	n	40
			221	13	2	n	
			23·3	1	1·1	0	
3	Staph. aureus. (Bacteriæmia)	…	540	n	31	0	
			54	14	4·5	0	
			5	1	0	0	
			0·5	0	0	0	
4	Bact. typhosum "O" 1. (From blood; enteric fever)	60	28,000	c	0	0	200
			2800	n	0	0	
			280	2·2	0	0	
			28	0	0	0	
5	Viridans streptococcus 1. (S.B. endocarditis)	60	220	n	n	0	
			33·4	9·7	n	0	
			14·1	2	16	0	
6	Viridans streptococcus 2. (S.B. endocarditis)	150	13·4	24	65	0	
			·13	2	5	0	
			1	0	0	0	
7	Viridans streptococcus 2. (After chemotherapy of patient)	160	79	13	11	0	
			8	1·7	0·3	0	
			1	0	0	0	
8	Br. abortus 1. (From blood; undulant fever)	35	76	72	79	n	<25
			7·6	3·6	13	n	
			0·7	0·3	1·5	0	
9	Br. abortus 2. (From blood; undulant fever)	20	44·6	34	37	60	40
			5·4	5	3	60	
			1·4	1·2	1·5	30	
10	Br. abortus 3. (From blood; undulant fever)	30	22·2	13·3	27·5	30	40
			3·3	3·3	2·5	0	

No.	Organism						
11	Br abortus 4 (From blood, undulant fever)	30	17 / 1 7 / 0 1	16 / 1 / 0	31 2 / 2 5 / 1 1	14 / 150 / 16 c	100
12	Br abortus 5 (From blood, undulant fever)	90	16 / 2 1	10 5 / 2	20 7 / 3 / 0	11 / 15 c	100
13	Br abortus 6 (From blood undulant fever)	20	14 / 1 / 0 1	16 / 1 / 0	73 / 2 / 0 7	c / u / u	100
14	Br abortus 7 (From blood undulant fever)	12	70 / 0 1	08 5 / 8 / 7	317 / 0 / 7	11 / 90 c	100
15	Bact typhosum 1 (From blood, enteric fever)	10	7 / 0 7	32 7 / 4	u / 317	18 c / c c	1600
16	Bact typhosum O'2 (From blood, pyrexia and pleurisy)	20	14 / 4 / 0 3	13 7 / 17 / 0 5	40 / 45 / 0	c c c	100
17	Bact paratyphosum 1 (From blood, enteric fever)	10	15 / 15 / 0 1	14 5 / 1 5	16 7 / 0 7	u / c o / o	100
18	Bact coli aerogenes (From blood, pyrexia, bacteriuria and bacilluria)	20	10 6 / 1 / 0 1	10 7 / 17 / 0 5	53 / 5 5 / 0 5	c o c c	100
19	Str pyogenes, type III (From recurring ulcer in the leg)	>30	18 / 8 / 0 1		u / 5 0	c c o o	80
20	Bact paratyphosum C (From blood, enteric fever)	15	50 / 5 / 0 5	44 / 15 / 1 1	15 / 17 / 0 4	c / 18 c / u	100
21	Staph albus 1 (From blood, ?S B endocarditis)	00	40 / 4 6 / 0 4	0 / 17 / 0 2	20 / 02 / 1 1	o / c o o	<10
22	Staph albus 2 (From blood ?S B endocarditis)	68	50 / 5 / 0	23 7 / 1 7 / 0 2	20 / 14 / 1 7	o / z o	<10

C, c = grades of confluent growth
u = mainly discrete colonies, but uncountable

for although *Bact. typhosum* " 0 " 1 was fully susceptible, *Bact. typhosum* " 0 " 2 was resistant.

Table II shows that there is no relationship between susceptibility and Gram-staining, nor is the susceptibility necessarily due to any specific agglutinins present in the patient's blood, for in the *Brucella* tests there was no inhibition, whether the agglutinins were 1 : 400 in titre or absent altogether. Again, a patient's blood did not inhibit the growth of his *Bacterium typhosum* although the agglutinin titre was as high as 1 : 1600.

Tests 6 and 7 show that an endocarditis *viridans* streptococcus was not rendered more susceptible to the bactericidal power of the blood by chemotherapy.

It will be observed that there was, in general, growth after 24 hours when presumably the survivors had time to multiply, except in the case of *Bact. coli* 1, in which an inoculum of 49 but not of 490 was completely sterilised, and *Bact. coli* 2, in which an inoculum of 23 but not of 221 perished. Fajerman (1937) states that the bactericidal power of the blood wanes after 3 hours at room temperature and earlier at 37° C. and von Haebler and Miles (1938) have shown that bacteria vary in their susceptibility to the bactericidal power of normal blood.

Discussion

For a rigorous test of the pathogen-selective culture method, the organisms used must be fully established as the infecting agent. In the series of cases examined there was, with two exceptions, no doubt of the role of the bacteriæmic organisms in producing the illness from which the patient suffered ; and a glance at the duration of the illness at the time the test was started shows that the infections were well established. The exceptions are the two cases of *Staph. albus* bacteriæmia, whose reality may be doubted in so far as the species isolated is a not uncommon contaminant of blood cultures. However, the organism was in both cases repeatedly cultivated from the blood. In one case a pure culture of 58 colonies and in the other a pure culture of 600 colonies was grown in plates of 1 c.c. of blood made immediately after withdrawal. During the time they were observed, neither of the patients showed cardiac signs of endocarditis, but in other respects their disease resembled subacute bacterial endocarditis. In all 21 instances, therefore, the organism tested was established as the infecting agent and the results indicate that there is no constant association between a lack of bactericidal power of the blood and susceptibility to infection by a given organism. The variations in bactericidal power observed appear to depend not so much on the immune state of the patient as the nature of the infecting organism.

It should be noted, however, that the technique employed is not strictly comparable with that of Solis-Cohen, who uses coagulable blood and holds the blood-bacterium mixture at 37° C. for 24 hours.

The difference between whole blood and defibrinated blood in this connection is probably insignificant. The difference in the periods of incubation, however, is important. It is contended that blood having the degree of bactericidal power exhibited in some of the tests recorded above after 2 hours' incubation *in vitro* (*i.e.* in conditions leading ultimately to a deterioration of the bactericidal power) would make a significant contribution to the resistance of the infected subject, in whom this degree of bactericidal power is presumably maintained for long periods. On the other hand, the survival of bacteria in the Solis-Cohen clot does not distinguish complete resistance of the inoculated organisms to the antibacterial action of the blood from a capacity of a few organisms to survive until the bactericidal power of the in-vitro clot has deteriorated. While therefore the technique may have an empirical justification, the unwarranted immunological assumption that the blood has little action on the infecting organism is not justified. The second assumption made by Solis-Cohen, namely that organisms of no ætiological significance are killed by the host's blood requires testing by a strictly quantitative technique like that employed in the above tests. It is probable that a number of the commensals usually encountered in mixed cultures from the mouth, nose, etc. will exhibit degrees of susceptibility to the bactericidal power of blood similar to those of the organisms tested (see, for example, von Haebler and Miles).

Summary

1. The bactericidal power of the blood of 21 patients was tested against the freshly isolated organisms causing the bacteriæmia. The marked bactericidal effect after 2 hours' incubation with the blood of the host throws great doubt upon the underlying assumption of the pathogen-selective culture method.

2. There is evidence that the susceptibility to the bactericidal power of the blood is a property of the bacterial species and that it may differ from strain to strain in the same species.

3. The presence of specific agglutinins is not constantly associated with heightened susceptibility of the infecting organism to the bactericidal power of the blood of the host.

4. In seven cases of undulant fever the blood of the host under the conditions of the test had no bactericidal power for the infecting *Brucella* strain.

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MORPHOLOGICAL CHANGES IN THE RED CELLS IN RELATION TO SEVERE BURNS

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In a study of the hæmatological changes in burns (Brown, 1944), it was shown that burns involving more than 15 per cent. of the body surface are frequently followed by a rapidly occurring moderately severe anæmia, the development of which is associated with a slight and variable increase in the mean corpuscular volume, increased erythrocyte fragility and increased plasma bilirubin and urobilin excretion. In the original publication only a preliminary report was made on the changes in size of the red cells. The results of further study of the material and findings obtained in the original investigation are now presented. These studies are concerned with the variations in mean corpuscular volume (M.C.V.), mean corpuscular diameter (M.C.D.) and mean corpuscular average thickness (M.C.A.T.), in relation to variations in osmotic fragility and the mechanism of production of the anæmia.

Methods

The three patients referred to here and the methods of estimation of hæmoglobin and of saline fragility have been described in the original report, to which reference may be made for full details.

Hæmoglobin was estimated photo-electrically, using carboxyhæmoglobin as the pigment. Saline fragility was estimated quantitatively, percentage lysis in each concentration of saline being obtained by comparison with a standard equivalent to 50 per cent. lysis, using a Duboscq colorimeter.

Red cell diameters were measured by microprojection on to millimetre-ruled graph paper at a magnification of 1000 diameters. Each red cell diameter recorded was the average of two measurements at right angles to each other. Evenly spread coverslip preparations stained by Leishman's stain were used and 400 cells were measured in each film.

Results

In the original study, blood films were prepared from all samples of blood obtained for hæmatological investigation. No significant alteration in the size or shape of the erythrocytes was apparent in patients mildly burned. In cases with severe burns it was evident, even from cursory inspection of stained films, that microspherocytosis was a prominent feature within a few hours of burning. It persisted

in gradually diminishing degree for 2-4 days after injury. Fragmentation of red cells was observed only in films made within a few hours of burning. In such cases small irregular portions of red cells apparently fully hæmoglobinated and measuring $0 \cdot 5$-$2 \cdot 0$ μ in diameter were numerous. In some instances a few ghost cells and non-hæmoglobinated fragments were also seen. In preparing the distribution curves illustrating changes in red cell diameter, fragmented cells were ignored as far as possible. These curves are not shown here, but the mean values for cell diameter are contained in the tables.

Case reports

Case 1 (no. 46 in original series). Female aged 10, 80 per cent. of body surface burned. This child sustained extensive third degree burns about 1 hour before admission to hospital. The findings in the 4-day survival period are recorded in table I.

TABLE I

Blood findings in case 1

	Time after burning (hours)							
	1·5	3·5	5	8	18	54	72	96
Hb. (g. per 100 c.c.) .	18·6	12·1	14·5	...	12·4	14·0	11·2	10·5
P.C.V. (per cent.) .	46·0	32·0	37·0	35·0	30·0	37·5	32·5	30·5
M.C.V. (c.μ.) . .	104	94	94	104	83	108	109	105
M.C.D. (μ) . .	6·23	6·56	6·52	6·78	7·15	7·75	7·87	7·75
M.C.A.T. (μ) . .	3·40	2·82	2·80	2·88	2·06	2·30	2·25	2·23
M.C.F. (per cent. NaCl)	0·436	0·440	0·424	0·422	0·400	0·373	0·328	0·324
Residual lysis (per cent.) *	15·0	10·0	10·0	8·0	5·0	3·0	0	0
Plasma † Hb (g. per 100 c.c.)	1·5	0·5	0·6	0·4	<0·1	0	0	0

* In this case lysis which persisted in concentrations of NaCl above 0·50 per cent.

† Hæmoglobinæmia and hæmoglobinuria were present in this case.

On admission there was evidence of hæmoconcentration. The M.C.V. was slightly above normal, although the average diameter was diminished. An increase in the M.C.A.T. was associated with a slightly raised median corpuscular fragility (M.C.F.) and with hæmolysis amounting to 15 per cent. in all concentrations of sodium chloride above 0·50 per cent. Hæmoglobinæmia and hæmoglobinuria were prominent features. During the period of survival the M.C.D. progressively increased, the M.C.A.T. and the osmotic fragility diminished, and the hæmoglobinæmia and hæmoglobinuria disappeared. Fragmented cells were numerous in the first two samples but rapidly disappeared thereafter. Microspherocytes were obvious in early films and, even in the last sample, 96 hours after injury, a few very deeply stained cells still remained.

Case 2 (no. 48 in original series). Female aged 7, 40 per cent. of body surface burned. This child was admitted to hospital with severe third degree burns about 3 hours after injury and the first samples of blood were obtained almost immediately (table II). She died three weeks later of agranulocytosis.

On admission there was evidence of hæmoconcentration. The M.C.V. was subnormal and this was associated with a reduction in diameter rather than in thickness. The M.C.F. was within normal limits and there was neither

visible hæmoglobinæmia nor hæmoglobinuria   At the 6th hour the spherocytosis had increased and the M C F. was found to be abnormally high   Thereafter osmotic fragility returned to normal and to subnormal, in association with an increase in the M C D and a reduction in the M C A T

<center>TABLE II</center>

<center>*Blood findings in case 2*</center>

	Time after burning (hours)							
	3	6	10	26	40	60	96	120
Hb (g per 100 c c)	18 7	12 6	10 7	12 2	12 0	12 0	10 1	9 5
P C V (per cent)	50 0	35 0	28 5	33 0	39 0	35 0	29 5	24 5
M C V (c µ)	75 0	86 0	80 0	98 0	03 5	06 5	98 5	87 0
M C D (µ)	0 75	6 27	6 39	7 50	7 63	7 01	8 08	7 75
M C A T (µ)	2 10	2 80	2 14	2 22	2 04	1 97	1 95	1 85
M C F (per cent NaCl)	0 424	0 400	0 394	0 404	0 385	0 390	0 368	0 352

In this case fragmentation of the red cells was not an obvious feature, and microspherocytosis was less obvious than in case 1   The change was, however, still visible in films 96 hours after burning

Case 3 (no 58 in original series)   Male aged 35, 40 per cent of body surface burned   Almost the entire burn in this case resulted in whole skin loss   The hæmatological findings are shown in table III   This patient survived, but was left with great disability owing to destruction of tissue in the limbs

<center>TABLE III</center>

<center>*Blood findings in case 3*</center>

	Time after burning (hours)						
	3	5	12	16	36	60	72	120
Hb (g per 100 c c)	19 0	10 1	20 4	13 2	17 5	16 9	14 0	12 4
P C V (per cent)	50 0	50 5	52 5	31 5	40 0	41 5	40 0	34 5
M C V (c µ)	98 0	98 0	94 5	91 0	102	81 0	95 5	103
M C D (µ)	6 77	6 67	7 15	6 92	7 06	7 24	7 49	7 66
M C A T (µ)	2 73	2 82	2 35	2 41	2 60	1 97	1 95	2 12
M C F (per cent NaCl)	0 476	0 448	0 454	0 444	0 406	0 380	0 360	0 340

Three hours after injury hæmoconcentration was a marked feature   The M C V was normal but the values for diameter and thickness indicated a significant degree of spherocytosis   The increase in M C A T was maintained throughout the first 36 hours and in the first 4 samples of blood (to 16 hours) there was a great increase in osmotic fragility   The M C F returned to normal and subnormal as the cell diameter increased and the M C A T fell to about 2 µ

In this as in the previous case fragmentation was not an obvious feature even in the first specimen of blood   Microspherocytosis was less marked than in the first case (case 46), and it was no longer seen after 36 hours

Discussion

The three patients described in this report are examples of very severe burns   All developed anæmia after the injury, and in one (case 1) hæmoglobinæmia and hæmoglobinuria were prominent features

Investigation of a large series of burns of different degrees of severity (Brown, 1944) has shown that anæmia of a hæmolytic type tends to follow those involving more than 15 per cent. of the body surface. A summary of the changes in M.C.V. was presented in the original report. These were so variable as to provide inconclusive evidence of definite alterations in cell size due to the injury. The reason is clear. The mean corpuscular volume is a function of diameter and thickness : it may be little altered if spherocytosis is produced at the expense of diameter. Such a change has been shown to occur after severe burns. The most severe injuries are associated not only with microspherocytosis but also with actual fragmentation of the red cells, intravascular hæmolysis and hæmoglobinuria. Less severe damage is associated with microspherocytosis alone, which is obvious in stained films and is further revealed by the rise in the M.C.A.T. It is associated with a demonstrable increase in osmotic fragility.

It has long been known that morphological changes occur in the blood of a burned patient, yet little information is to be obtained from modern works on hæmatology as to the nature of these changes and their ætiology. Whitby and Britton (1944) state that "the mechanism of hæmolysis in severe burns has not been fully elucidated, but it is supposed to be due either to secondary infection, or more probably to absorption of hæmolytic protein cleavage products". Wintrobe (1942) limits himself to the statement that "Severe hemolytic anemia with hemoglobinuria is said to occur . . . sometimes following *extensive burns*".

In reviewing the literature on the effect of heat on the blood and of severe burns on the patient, no reference has been found indicating that hæmolytic protein cleavage products may be responsible for the production of hæmolytic anæmia after such injuries. On the other hand, there is good evidence that heat is responsible for certain morphological changes in the red cells whereby these are rendered unduly susceptible to the physiological trauma of the circulation and intravascular hæmolysis occurs.

The earliest studies of changes in the red cells in burns were undertaken in an attempt to explain death from burns shock. Schultze (1865) observed that crenation and fragmentation followed exposure of the blood to temperatures of 51-52° C. Similar findings were recorded by von Lesser (1880), Silbermann (1890), Burkhardt (1904-05) and Helsted (1906). Experimental burns in animals were found to be followed by similar changes in the blood within a short time of injury (von Lesser, 1880 ; Markusfeld and Steinhaus, 1895 ; Pfeiffer, 1905). Hæmoglobinæmia and hæmoglobinuria were observed in animals burned by scalding and in animals transfused with blood from a burned animal. Isaacs *et al.* (1924-25) confirmed the morphological changes described in heated blood, and observed that red cells heated above 50° C. showed increased osmotic fragility. Locke (1902)

recorded fragmentation of red cells in two fatal cases of burns within $1\frac{1}{4}$ hours of injury.

These experimental and clinical findings have recently been confirmed by Shen and Ham (1943), who demonstrated the occurrence of intravascular hæmolysis with hæmoglobinæmia and hæmoglobinuria in several cases of severe burns. In some cases the red cells showed increased osmotic fragility which was associated with microspherocytosis as estimated by inspection of stained films. It has previously been shown (Brown) that burned patients, roughly in proportion to the severity of the burn, are liable to become anæmic within a week of the injury and that the development of the anæmia is associated with a slight and variable increase in the M.C.V. and increased osmotic fragility of the red cells. Plasma bilirubin and urinary excretion of urobilin are increased (Anderson and Semeonoff, 1944). The greatest change in fragility was found to occur in association with hæmoglobinæmia and hæmoglobinuria in patients with extensive and deep burns. In the present investigation it has been shown that these alterations are accompanied by a demonstrable increase in the mean corpuscular average thickness of the red cells. The impression of microspherocytosis obtained from inspection of stained films (Shen and Ham) has thus been confirmed.

The fundamental cause of the change in the red cells leading to hæmolysis is probably the direct action of heat. When blood is heated rapidly to 51-55° C. and then immediately cooled to 37° C., the changes produced are exactly those which have been shown to occur in the burned patient. In addition, these changes are independent of the fluid in which the cells are suspended (Shen and Ham). These authors have also shown that blood which has undergone such changes is abnormally susceptible to mechanical trauma artificially produced. The probable mechanism of hæmolysis in burns thus becomes increasingly evident.

Conclusions

Investigation of the morphological changes occurring in the red cells of three very severely burned patients has shown that fragmentation of the red cells and microspherocytosis occur within a few hours of the injury. Microspherocytosis was seen in stained films and was shown to be associated with an increase in the mean corpuscular average thickness. Fragmentation of the red cells was most obvious in the most severely burned patient and was associated with hæmoglobinæmia and hæmoglobinuria.,

The morphological changes occurring in the red cells in burns can be attributed to the direct action of heat on the cells. The maximum effect is immediate fragmentation and destruction. If the damage is less severe microspherocytosis is produced and the affected cells are unduly susceptible to the physiological trauma of the circulation.

Depending on the magnitude and rate of hæmolysis, hæmoglobinæmia and hæmoglobinuria may occur.

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A HISTOPATHOLOGICAL STUDY OF SMALL LUNG-WORM INFECTION IN SHEEP AND GOATS WITH SPECIAL REFERENCE TO MUSCULAR HYPERTROPHY OF THE LUNG *

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Lungworm infection is almost universal among sheep and goats in the north-west of China but is not considered to be a serious source of economic loss. Heavy infections are often harboured by apparently healthy sheep which show no clinical evidence of infection. It is generally of secondary rather than primary importance, its effects being supplementary to those of nutritional disturbances and other parasitic infections. In addition, it predisposes the affected pulmonary tissue to secondary bacterial invasion. Since histological studies have revealed several features of interest, this report seems warranted.

Material and Methods

The lungs of 90 sheep and 10 goats were collected from the slaughter house for examination. The age was only noted in lambs and kids under one year. As a rule blocks were taken from both consolidated and apparently normal areas. They were fixed in 10 per cent. formalin. Paraffin sections were cut from 5 to 10 μ in thickness, and stained as a routine with Harris's hæmatoxylin and eosin. In addition, van Gieson's stain, Heidenhain's azan stain, Weigert's resorcin-fuchsin stain for elastic tissue and Weigert's differential stain for fibrin were used from time to time. Serial sections were studied in several cases.

Results

Gross examination

The presence of small lungworms (*Protostrongylus sp*) was indicated by raised greyish nodules and cone-shaped areas of consolidation ranging from 1 to 2 cm. in diameter, just beneath the pleural surface. They were usually located in the dorsal border of the posterior lobes and it is worth noting that they were not met with in the interior of the lung. Smears from the cut surface usually revealed segments of adult worm, ova at different stages of development and larvæ of the Protostrongylus group. On opening the bronchi small

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lungworms were found in varying numbers, both in the peripheral part of the bronchial tree and in the pulmonary tissue. Large lung-worms were sometimes found in the larger bronchioles and branches of bronchi as a mixed infestation. Probably they have nothing to do with the raised nodules mentioned above. Cases with mixed infestation, however, were not included in the present study.

Microscopical examination

Normal histology. In order to appreciate the morbid changes, it is advisable to give a brief general description of the normal lungs of the sheep and goat. The pulmonary tissues of these two animals are so similar that one description will serve for both. The visceral pleura is very thin, consisting of a layer of mesothelium and a small amount of subpleural connective tissue. There is no smooth muscle in the pleura or interstitial tissue. Under normal conditions there are no prominent blood and lymphatic vessels. Only a few lymphoid cells are found in the pleura at its junction with the pulmonary tissue. The peribronchial and perivascular tissues and the interlobular septa also contain small accumulations of lymphoid cells. The bronchi and bronchioles are lined by ciliated columnar epithelium. The muscular coat is rather thin and its thickness decreases from above downwards. The respiratory bronchioles are lined by a layer of low cuboidal epithelial cells devoid of cilia; the muscular coat is very thin but easily seen. In the rings surrounding the openings of large units of air spaces there are a few very thin muscle fibres. After careful search one can always discern some muscle fibres—thin fibres only—in the wall of the ductules. These fibres sometimes extend from the proximal to the distal end of the ductules but never down to the wall of the air sacs. The alveolar septa are very delicate structures, consisting of two layers of non-nucleated epithelial cells and a capillary network.

Morbid histology. (1) The pleura over the worm nodule (area of consolidation) is usually slightly or markedly thickened. Dilated blood and lymphatic vessels are found in a few cases. In the sub-pleural tissue lymphoid cells were increased in number, even with the formation of lymph nodules and germ centres. No muscle fibres are seen in the pleura or interstitial tissue.

(2) The larger bronchi and bronchioles are usually empty or contain only a few larvæ in mucus. The mucous membrane appears normal except for an increase of mucus-secreting cells and occasional congestion. The bronchial glands are mostly normal, but in a few cases they are dilated and distended with pink-staining secretions. A larva was once noticed in the lumen of a bronchial gland. The muscular coat is generally thin and only rarely hypertrophied. The adventitia is usually infiltrated by lymphoid cells. In many cases the infiltration is extreme, with formation of lymph nodules and germ centres. The terminal and respiratory bronchioles show as a rule some degree of muscular hypertrophy, at times very marked. The mucous membrane appears thickened as a result of lymphoid cell infiltration beneath it, while the adventitia also is infiltrated by a small number of lymphoid cells. The lumina are sometimes blocked, partially or

completely, by adult small lungworms or by mucus containing larvæ
The ductules show varying degree of muscular hypertrophy   The
muscle in the rings surrounding the air spaces is very prominent
At times the muscle of the ductules is so much hypertrophied that
it is even thicker than that of the respiratory bronchioles   Muscle
is not seen to have extended from the ductules to the walls of the air
sacs   Adult worms, ova and larvæ are also found in the ductules
and air sacs   The alveolar septa are thin as a rule, though sometimes
thickened as a result of infiltration by small round cells and poly-
morphonuclear leucocytes or by proliferation of septal cells

(3) The consolidated areas of lung present several features of
special interest   (a) In a few cases the air sacs are packed with
undeveloped ova and adult worms but the alveolar walls appear
completely normal   there is no reaction at all towards the ova
Muscular hypertrophy is absent or very slight   Elsewhere the ova
are in different stages of development and likewise without tissue
reaction   Lymphoid cell infiltration is present in the subpleural,
peribronchial and perivascular tissues in varying degree but is seldom
extreme   (b) In most cases there is a macrophage reaction towards
the developing embryos and larvæ filling the air spaces   Most of the
macrophages are the alveolar phagocytes of septal origin   Very
often they spread over the surface of the ova, the developing embryos
and larvæ, forming rudimentary foreign body giant cells   Sometimes
they gather around a larva, with formation of a pseudo-tubercle
Free foreign body giant cells and macrophages are also seen in the
air sacs   Pink staining albuminoid material is present in the alveoli
in a few cases   Precipitated fibrin, as described by Taliaferro and
Sarles (1939), is occasionally seen on the surface of larvæ and developing
ova   Larvæ surrounded by macrophages and precipitated fibrin are
rather thin and seem to be stunted in growth   The walls of the air
sacs appear to be thickened and infiltrated by small round cells and
polymorphonuclear leucocytes   (c) Leucocytic exudate is occasionally
observed in the air sacs, due apparently to bronchopneumonia following
secondary bacterial invasion   Eosinophils, contrary to expectation,
are rarely found

Areas of collapse are often found   They are sometimes infiltrated
by lymphocytes or polymorphonuclear leucocytes or both   They
may contain ova or larvæ at various stages of development   The
muscle in the collapsed pulmonary tissue may show marked
hypertrophy or none at all   In the neighbourhood of the collapsed
and consolidated areas, the alveoli and terminal bronchioles are often
dilated (compensatory emphysema) and the walls of the air sacs may
be broken down

General considerations

(1) *Localisation of the nodules*   The worm nodules, usually cone-
shaped, lie immediately beneath the pleura   They are located as a

rule in the dorsal border of the posterior lobes, are seldom found elsewhere and never in the interior of the lungs. It is surmised that the better ventilation afforded by the subpleural pulmonary tissue may be the determining factor for this predilection.

(2) *Tissue reaction.* In all probability the *adult worms* do not produce any seriously harmful effect on the host, as they do not call forth any cellular reaction, nor any humoral reaction as evidenced by the absence of precipitated fibrin on the surface of the cuticle. Whatever disturbances there are, are probably due to mechanical irritation or obstruction when worms are present in large numbers. There is increased production of mucus by the bronchial mucosa. The worms seem to live on good terms with the host. Dead worms are not found either in the bronchioles or in the pulmonary tissue. It is likely that when dead they are expelled by the peristaltic movements of the bronchial muscles. As a corollary we are inclined to think that living worms might strive to move against the direction of the peristaltic movements of the muscle, and if this is true, it may explain why they are found mainly in the peripheral parts of the lung. After examining a large number of specimens, we were able only exceptionally to find a few dead worms embedded in masses of lymphoid tissue.

The *ova*, especially the unsegmented ones, do not produce any reaction in the host, even when there is cellular reaction towards the larvæ and developing embryos. There are a few cases—confined to lambs—in which ova and larvæ together produce no reaction in the host. In certain instances, however, the ova are attacked by macrophages—usually septal cells—which are in close contact with the surface of the ova and form rudimentary foreign body giant cells. As a rule only a small number of ova are so attacked, and then the majority of the larvæ are attacked also.

The *larvæ* are most often attacked by macrophages, which spread over the surface like a sheath, or may even form a pseudotubercle enclosing the attacked larvæ. In addition the cuticle of the larvæ is sometimes covered by a layer of precipitated fibrin. Larvæ so surrounded appear thinner than normal: they are apparently somewhat immobilised and stunted in growth. The presence of a cellular reaction and precipitated fibrin may be regarded as evidence of a defensive mechanism.

It is evident from the above that there may or may not be tissue reaction towards ova or larvæ. With regard to this inconsistency two factors may be considered: the age and the immunological state of the affected animal. Cellular reaction is often absent in young animals, but we are inclined to think that age *per se* cannot be the sole cause and that the immunological state must be taken into consideration. Taliaferro and Sarles have definitely shown that the immunological state determines the histological picture in various tissues of rats experimentally infected with *N. muris*, and it would

seem that a similar state of affairs might be present in the pulmonary tissue of sheep and goats in regard to small lungworm infection.

(3) *Muscle in the normal lung.* (*a*) *Pleural and interstitial tissues.* In the pulmonary pleura and interstitial tissue no muscle fibres are found in any of the sections examined. (*b*) *Musculature of the air spaces.* The general arrangement of the muscle is similar to that described by Baltisberger (1921) and by Engel and Newns (1939) for human lungs, but the amount is less. There are some fibres in the rings surrounding the openings of the larger units of air spaces, as for instance at the proximal ends of the ductules, but they are very thin as compared with the thick bundles described by Baltisberger for human lungs. Near the periphery only occasional muscle fibres can be recognised, but at times they can even be found near the peripheral end of a ductule. We shall see later that single fibres must have been present, if not easily discernible, over the whole length of the ductule to produce the hypertrophy seen in pathological lungs.

(4) *Muscular hypertrophy in pathological cases.* As in the normal lung, no muscle fibres are observed in the pleura or interstitial tissue. The musculature of air spaces, however, has an important role to play in infested lungs. In this respect our findings conform with those of previous observers upon human lungs (Engel and Newns) but the degree of hypertrophy is even more marked. The predominance of muscle in the rings surrounding the air spaces is easily recognised, even in hæmatoxylin and eosin-stained sections. Thick bands are usually seen, extending from the proximal to the distal end of the ductules, but no muscle fibres are seen in the walls of the air sacs. It is of interest to note that the lumina of the ductules and respiratory bronchioles are often partially obstructed by worms or mucus.

Musculature of respiratory bronchioles. Whenever the muscle of the ductules is hypertrophic the muscle of the respiratory bronchioles is similarly affected, the muscle of these two structures being directly continuous. It is not infrequently observed that the muscle of the respiratory bronchioles is less hypertrophic than that of the ductules.

Musculature of bronchioles. This is less frequently involved in the hypertrophy. Only in a few instances are thick muscle bands present in the wall. It was of interest to note that where the bronchiolar muscle is hypertrophic the muscle of the respiratory bronchioles and ductules is similarly affected. Obstruction is always present, albeit incomplete. It is thus apparent that there must be some relationship between muscular hypertrophy and obstruction in the bronchiolar system.

It must be noted that this muscular hypertrophy does not affect the whole lung to the same extent and degree. As a rule hypertrophy is greatest in the consolidated areas. Pulmonary tissue remote from these areas shows little if any muscular hypertrophy. The hypertrophy

is more marked in the periphery of the bronchiolar system—ductules and respiratory bronchioles—than in the larger tubes.

When the hypertrophy is located in a collapsed area, muscular tissue is the only prominent structure under the microscope, and the term "muscular cirrhosis" (von Stössel, 1937) might well be applied to describe the pathological picture.

(5) *The cause of the muscular hypertrophy.* With regard to muscular hypertrophy in human lungs Engel and Newns suggested two possibilities : it might be a congenital anomaly or it might be caused by some pathological stimulus. They could give no direct evidence as to the truth of the first possibility and our findings also fail to provide support for this theory. We are inclined to favour the second possibility—some pathological stimulus. It is self-evident that this stimulus may differ under different disease conditions, and as a result the muscular response will not be similar in distribution and degree. Attention may be drawn to the fact that in our cases the muscle of the pleura and interstitial tissue is unaffected, although in Engel and Newns's case of chronic rheumatic carditis it was a prominent feature. The latter was due to obstruction in the pulmonary circulation, while in our series the hypertrophy seen in the bronchioles and ductules is due to obstruction in the bronchiolar system. We are inclined to interpret this difference as dependent on the nature of the primary disease process rather than on variation in the animal species. In our cases another possible contributory factor worth considering is that the wriggling movement of the worms and larvæ may have exerted a strong enough influence to cause hypertrophy of muscle.

We know from experience that both striated and smooth muscle fibres are capable of rapid hypertrophy under the stress of functional demand. In pathology we have numerous examples of hypertrophy of smooth muscle in the walls of hollow organs as a result of obstruction in the lumen, especially when the obstruction is intermittent or incomplete. In lungworm infection the bronchioles are as a rule partially obstructed by worms or mucus, and we might reasonably expect to find muscular hypertrophy distal to the site of obstruction. If obstruction is rapidly produced and complete the result will be collapse. If complete obstruction is superadded in an area previously affected with partial obstruction and muscular hypertrophy, the result will be a combination of collapse and muscular hypertrophy—the so-called muscular cirrhosis of the lung.

SUMMARY AND CONCLUSIONS

1. The small lungworm of sheep and goats lives in the bronchioles, alveoli and pulmonary parenchyma. It deposits its eggs to hatch in the alveoli.

2. The presence of small lungworm is indicated by raised greyish

nodules, 1-2 cm. in diameter, beneath the pleura. The nodules are located as a rule in the dorsal border of the posterior lobes; so far they have not been found in the interior of the lungs.

3. The cellular reactions are macrophagic, lymphocytic and eosinophilic. In most cases there is a macrophage reaction around both larvæ and ova, with formation of foreign body giant cells and even pseudo-tubercles. Precipitated fibrin is observed on the surface of the attacked larvæ and ova. In the lamb, however, there is sometimes no cellular reaction at all. The difference is presumably due not only to the age of the animal but also to its immunological state. A small number of lymphoid cells is normally present in the subpleural region, interlobular septa and peribronchial and perivascular tissues, but in lungworm-infected lungs these cells are greatly increased in these situations, with formation of large lymph nodules and germinal centres. Eosinophilic reaction, contrary to expectation, is usually absent. With secondary bacterial invasion the cellular exudate assumes the type characteristic of ordinary bronchopneumonia.

4. In the normal lungs of sheep and goats muscle fibres are not seen in the pleura or interstitial tissue, while in the walls of the air spaces only fine muscle fibres are found : there are no thick bundles. Since, in the pathological conditions described, we have observed hypertrophic muscle bundles in the ductules, even towards the periphery, we may assume that, normally, fine fibres must have been present here to render possible the hypertrophy seen.

5. This muscular hypertrophy is the result of obstruction in the bronchiolar system. If the obstruction is slowly produced and incomplete, there is usually hypertrophy of the musculature of the terminal bronchioles and ductules ; if rapidly produced and complete, the result is collapse without muscular hypertrophy. If complete obstruction is superadded to incomplete, the resulting lesion is a combination of hypertrophy and collapse—the so-called muscular cirrhosis of the lung.

6. The wriggling movement of the worms and larvæ may be considered as a contributory factor in the causation of the muscular hypertrophy.

7. Compensatory emphysema is usually associated with consolidation and collapse of the lung parenchyma.

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in the yolk sac of developing eggs. This method, again, was impracticable in the day to day testing of each small batch, but it was intended to devise such a test for the bulked vaccine of 5-10 litres which represented the pooling of many daily lots prior to bottling. The ending of hostilities caused the further production of the vaccine to be abandoned before this test could be developed.

In the early days, before experience had been gained, the daily batches of vaccine showed wide fluctuations in rickettsial content and it was essential to evaluate them individually if the pools made up from them were not to be significantly diluted by inferior lots. These daily batches were therefore controlled by the following methods.

1. Control of the lungs used for vaccine production.
2. Control of the finished batches of vaccine.

    (a) Rickettsial count.
    (b) Antigenic content of vaccine as judged by complement fixation.

CONTROL OF THE LUNGS USED FOR VACCINE PRODUCTION

In order to make certain that the material being used for vaccine production was of a sufficiently high standard, the rickettsial content of the cotton rat lungs had to be estimated.

The method of infecting the cotton rats and of handling their lungs has been described by Buckland *et al.* Briefly, batches of about 72 cotton rats were infected each day. These rats, arbitrarily classified into 6 groups of 12, were inspected daily for the first 3 days and any dead animals discarded. Thereafter they were inspected every $1-1\frac{1}{2}$ hours both day and night and all dead animals removed and their lungs collected. As they became available, the lungs were stored in bottles packed in solid CO_2 so that the lungs of any batch should be available for processing together. On the 9th day after inoculation, or when 80 per cent. of the rats of any batch were dead, the survivors were killed and their lungs collected.

Each day at least one sample of lung from each group of 12 rats where deaths had occurred was taken for the preparation of impression smears.

Staining of impression smears

While investigating the staining properties of basic fuchsin and methylene blue, it was found that treatment of a heat-fixed smear with N/1 HCl considerably enhanced the staining of rickettsiæ with either dye.

Monochrome staining method. This method was quick and easy and was used for regular routine purposes. A light impression smear of the cut surface of the lung was fixed by gentle heat. The smear was then treated with N/1 HCl for

two minutes, washed and stained with 1 : 2000 polychrome methylene blue for 30–60 seconds. The methylene blue was polychromed as a 1 per cent. solution with 1 per cent. sodium carbonate by heating at 56° C. for 45–60 minutes. On cooling, the solution was neutralised with N/1 HCl, filtered and stored. Before use it was diluted 1 · 20.

Differential staining method. For special purposes another method was used which had the great advantage that it stained the rickettsiæ a different colour from the cells. It was only satisfactory, however, in expert hands, because the differentiation was exceedingly critical and the smears, which had to be prepared with great care, had to be of uniform thickness and free from blood. Suitable smears were fixed by gentle heat and treated for 10–15 seconds with 10 per cent. HCl. They were washed and stained for 2 minutes with boiling carbol fuchsin (Ziehl Neelsen carbol fuchsin diluted 1 : 5), again washed, and differentiated and counter stained with 1 per cent. malachite green (Revector) for 30–60 seconds. The exact staining time for the malachite green varied considerably with different batches. Rickettsiæ stained a deep reddish purple against a greenish blue background of cytoplasm and nucleus (fig. 1).

The method was also applicable to sections fixed in formol-Müller. In this case, however, the section, after having been stained with boiling carbol fuchsin, was decolorised with 5 per cent. citric acid for 10–15 seconds and then counter stained with malachite green for 10–25 seconds (fig. 2).

Assessment of lung smears

Stained impression smears were examined under a 1/12th inch oil immersion objective and ×6 ocular. An ideal smear was given an arbitrary index of 10, assessed in this way. Since the intracellular rickettsiæ were considered the most important source of antigen in the cotton rat lung, this attribute was awarded 8 points. The degree of parasitisation was established by counting 20 consecutive mononuclear cells in different parts of the smear and recording the number which contained rickettsiæ. This number was multiplied by 0·4. The extracellular rickettsiæ were awarded a possible 2 points, estimated by giving a plus value (maximum 4+) and multiplying this value by 0·5. For example :—

Intracellular rickettsiæ in 12 out of 20 mononuclears = $12 \times 0·4 = 4·8$
Extracellular rickettsiæ +–+–+ = $3 \times 0·5 = 1·5$
$$\overline{6·3}$$

$$\text{Index} = 6$$

In the majority of the cotton rat lungs which were suitable for inclusion in the vaccine, the index was between 5 and 8, and the rickettsial content of the smears of individual lungs gave good evidence of the expected rickettsial content of the finished vaccine. It was concluded that with few exceptions the lungs from batches of rats in which 50 per cent. or more died on or before the 6th day were the most suitable for vaccine manufacture, provided that by the 5th day the lungs of rats dying showed massive consolidation and an index of 6. Table I shows a detailed assessment of the lung smears from an early batch (FV 37) which provided a vaccine with a poor

rickettsial content. Table II shows the assessment of a later batch (FV 131) which provided a more satisfactory vaccine.

TABLE I

Analysis of lungs used in the manufacture of vaccine batch FV 37 (low rickettsial count)

FV 37		Number of cotton rats inoculated, 72							
Day after inoculation	Cotton rats dying		No. of lung samples	Lung indices					Average lung index
	No.	Cumulative percentage							
1-3	5	7·0	...	...	...	...	...	...	...
4	0	...	...	...	...	...	...	...	...
5	1	8·4	1	4	...	...	...	...	4·0
6	7	18·2	5	7	4	5	5	3	4·8
7	8	29·4	5	4	7	4	5	4	4·8
8	13	47·6	5	6	6	5	3	7	5·4
9	7	57·4	2	4	5	...	...	...	4·5
Surviving on 9th day	31	Rickettsial count of vaccine, 80×10⁶/ml.							

TABLE II

Analysis of lungs used in the manufacture of vaccine batch FV 131 (high rickettsial count)

FV 131		Number of cotton rats inoculated, 372													
Day after inoculation	Cotton rats dying		No. of lung samples	Lung indices											Average lung index
	No.	Cumulative percentage													
1-3	31	8·3	...												...
4	12	11·6	5	8	4	7	5	7							6·2
5	72	31·0	7	6	8	8	8	8	6	6					7·1
6	145	70·0	4	9	8	7	8								8·0
7	68	88·5	11	8	8	9	7	7	2	7	8	9	9	8	7·5
8	14	92·0	11	4	5	5	8	6	6	7	5	6	5	7	5·8
Surviving on 9th day	30	Rickettsial count of vaccine, 257×10⁶/ml.													

CONTROL OF THE FINISHED BATCHES OF VACCINE

Determination of the number of rickettsiæ

The rickettsial content was estimated by counting the stainable rickettsiæ. A small amount of the vaccine was mixed with an equal volume of a formalin-killed *Cl. welchii* suspension of accurately

STANDARDISATION OF SCRUB TYPHUS VACCINE
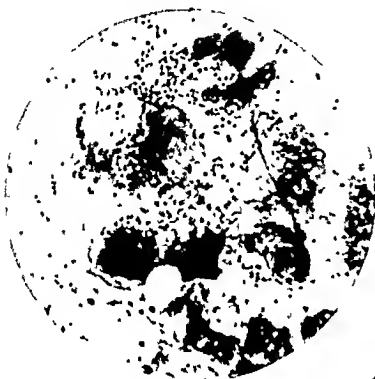
FIG. 1.—*Rickettsia tsutsugamushi.* Cotton rat lung impression smear. Carbol-fuchsin and malachite green. ×1250

FIG. 2.—*Rickettsia tsutsugamushi.* Section of cotton rat lung, fixed in formol Muller. Carbol-fuchsin and malachite green. ×1250.
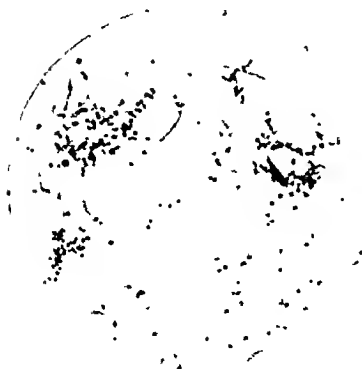
FIG. 3.—*Rickettsia tsutsugamushi.* A drop of the cotton rat lung vaccine. Carbol-fuchsin and malachite green. ×1250.

Complement fixation

Smadel (1944, personal communication) has devised a method of estimating the potency of epidemic typhus vaccines by using the vaccine as the antigen in a complement fixation test with specific sera of known titre. The potency of the vaccine was judged by the extent to which it could be diluted and still fix complement specifically. Vaccines do not give high values when tested in this way, because the antigen is so poorly dispersed. Thus a suspension of *R. prowazeki* is a weaker antigen in the complement fixation test than its soluble antigen, even though the rickettsial suspension contains a greater amount of specific antigen. The scrub typhus vaccine was very difficult to judge by this method because it was not a purified vaccine, and the lung debris resulted in an anti-complementary level only slightly below the antigenic level. It was therefore essential to design a very finely balanced test.

The sensitivity of the test could be increased by reducing the number of M.H.D. of complement used, and the extreme of 1 M.H.D. was found to be practicable provided that the complement was accurately titrated in the presence of the antigen, and provided that the specific and control antisera could be used diluted well above their own anti-complementary levels.

The test could have been made still more sensitive by primary fixation at +4° C. for 18 hours instead of at 37° C. for one hour, but unfortunately the non-specific fixation of the lung debris was notably increased by prolonged fixation in the cold.

Technique

Antigen. The vaccine to be tested was centrifuged at 12,000 r.p.m. for 30 minutes, the supernatant was discarded and the deposit was resuspended in an equal volume of saline. This was necessary, as the formalin present in the vaccine would have been, of course, strongly anti-complementary. Fulton and Joyner have shown that all the specific complement-fixing antigen of cotton rat lungs infected with *R. tsutsugamushi* is sedimentable with the rickettsiæ. We have confirmed this, and have found that supernatants of vaccines centrifuged at high speed and dialysed free of formalin do not fix complement specifically.

Sera. The specific serum was a pool derived from guinea-pigs convalescent from scrub typhus. As a control, serum from a guinea-pig convalescent from epidemic typhus was used. The complement-fixing titre of the specific serum was 1 : 320. Both sera were used in this test at 1 : 40, at which dilution neither was anti-complementary. They were inactivated at 56° C. for 30 minutes immediately before use.

Sensitised cells. The sheep cell suspension was approximately 7·5 per cent. and was accurately standardised by a colorimetric estimation of the hæmoglobin. The standardised suspension was sensitised by the addition of an equal volume of saline containing 5 M.H.D. of hæmolysin.

Titration of complement. A rack was set up with 5 rows of 10 tubes. Into each tube 0·25 ml. of saline was measured. In a separate set of 10 tubes was prepared a series of complement dilutions from 1 : 15·1 to 1 : 62·5. The dilutions were so arranged that the lowest complement dilution contained

0 0166 ml of guinea pig serum in each 0 25 ml , and each subsequent dilution reduced this amount by 0 0014 ml Of each complement dilution, 0 25 ml was pipetted into the appropriate tube of each of the 5 rows To each of the 10 tubes in row 4 were added 0 25 ml of the antigen diluted 1 8 Similarly to all tubes in rows 3, 2 and 1 were added 0 25 ml of antigen diluted 1 4, 1 2 and 1 1 respectively To each of the 10 tubes of row 5 were added 0 25 ml of saline The rack was shaken and the tubes incubated for 1 hour in a water bath at 37° C 0 25 ml of the sensitised sheep cell suspension was then added to each, and the tubes were returned to the bath for a further 30 minutes Readings were taken and were recorded as 4, 3, 2, 1, tr and 0, where 4 represented no hæmolysis and 0 complete hæmolysis The results of this titration gave the exact minimal hæmolytic dose of complement in the presence of each of the antigen dilutions under the conditions employed for the test

The main test A rack containing two rows of 5 tubes was set up Into tube 1 of each row were placed 0 25 ml of undiluted antigen, and an equal volume of a complement dilution containing 1 M H D of complement for that antigen dilution as determined in the preliminary titration Similarly tubes 2, 3 and 4 of both rows received 0 25 ml of antigen diluted 1 2, 1 4 and 1 8 respectively, and 0 25 ml of the complement dilution, providing exactly 1 M H D for the appropriate antigen dilution Tube 5 of each row received 0 25 ml of saline and 0 25 ml of complement dilution, providing 1 M H D of complement titrated without antigen These tubes served as the serum controls To all five tubes of the first row was added 0 25 ml of 1 40 specific antiserum, and to all five tubes of row 2, 0 25 ml of 1 40 control antiserum After shaking, the tubes were incubated for 1 hour in a water bath at 37° C 0 25 ml of sensitised sheep cells was then added, the tubes were returned to the water bath for a further 30 minutes and then read

Results

The power of the vaccines to fix complement was found to be correlated with their rickettsial content Table IV shows the titre of

TABLE IV

Complement fixing titres of some representative batches of vaccine compared with the rickettsial counts

Vaccine batch no	Rickettsial counts (millions/ml)	Vaccine dilutions			
		1 1	1 2	1 4	1 8
*H 6	309	†4	4	4	2
FV 5	65	4	3	0	0
FV 14	15	4	0	0	0
FV 19	31	2	tr	0	0
FV 33	164	4	4	4	tr
FV 37	80	4	4	2	0
FV 73	106	4	4	2	0
FV 90	205	4	4	4	2
FV 131	257	4	4	4	3

* This vaccine was made on a small scale at the National Institute for Medical Research
† The degree of fixation is expressed by one of six symbols —4 3 2 1 tr 0 4 signifying complete fixation and 0 no fixation

some representative batches of vaccine with the rickettsial counts in each case As fears had been expressed initially that a proportion of

the available antigen was being lost during the different stages of production, the complement-fixing titres of some of the vaccine batches were compared with samples of fresh lung tissue selected from the batches prior to processing.   A comparison of these titres was reassuring and showed that there was very little loss of antigen (table V).

<div align="center">TABLE V</div>

Complement-fixing titres of fresh lung suspensions compared with the titres of the appropriate finished vaccine

Batch nos.		Antigen dilutions				
		1:1	1:2	1:4	1:8	1:16
FV 14	Fresh lung suspension	*4	2	0	0	0
	Vaccine . . .	4	0	0	0	0
FV 37	Fresh lung suspension	4	4	4	tr	0
	Vaccine . . .	4	4	2	0	0
FV 33	Fresh lung suspension	4	4	4	4	0
	Vaccine . . .	4	4	4	tr	0

* The degree of fixation is expressed by one of six symbols: 4, 3, 2, 1, tr, 0, 4 signifying complete fixation and 0 no fixation.

DISCUSSION

The potency tests which were used to control the quality of the vaccine did not attempt to assess the value of the vaccine as an immunising agent in man, for only a field test could establish that. What was attempted was to raise the standard of the mass-produced vaccine to the level found to be possible when it was made as a research project.   Vaccine made at the National Institute for Medical Research contained approximately 300×10^6 stainable rickettsiæ per ml. when counted by the routine method (with a probable real value of 900×10^6/ml.), and it would fix complement when diluted 1:8. The mass-produced vaccine at first fell short of this standard, but as experience was gained it improved, so that latterly it closely resembled the vaccine made on a small scale.

SUMMARY

During the large scale manufacture of a scrub typhus vaccine from cotton rat lungs, it was necessary to find some method of standardising the batches of vaccine produced.   Owing to the peculiar difficulties of the process it was not possible to devise a direct test of the immunising potency and so it was decided to depend on an estimate of the rickettsial content.

The rickettsiæ in the cotton rat lungs were assessed by examining stained impression smears, and calculating a numerical value based

on the degree of parasitisation of the susceptible cells and on the number of extracellular organisms.

The rickettsial content of the vaccine was estimated by a direct counting method, and also by measuring the amount of specific complement-fixing antigen.

New staining techniques were used both in the routine preparation of impression smears and in the method used for enumerating rickettsiæ in the vaccine.

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MICROBIC DISSOCIATION IN *C DIPHTHERIÆ* BY THE PRODUCTION OF DAUGHTER COLONIES

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With a comment by J W McLeod

(PLATE LIII)

PREISZ (1903 04) was one of the first to observe the formation of secondary or daughter colonies in growing cultures of *C diphtheriæ* Eisenberg (1906) described them in many species of bacteria In growing cultures kept long at room temperature, Trautmann and Dale (1910) saw that delicate basic colonies might give rise to secondary growths with all the characteristics of *C diphtheriæ* These bred true end did not reproduce on subculture the delicate form of the mother colonies Bernhardt (1915) plated an old broth culture of *C diphtheriæ* and noted that daughter colonies developed on one of two colonial variants—a small transparent colony with indented edges

The secondary growths described by these and other authors appear to have arisen from an effort at survival on the part of certain cells in dying cultures, they differed, therefore from those which arise as a feature of actively flourishing strains in a dissociative phase of their life history McLeod end his co workers (Anderson *et al* 1931) described smooth, intermediate and rough colonial forms as *mitis, intermedius* and *gravis* types, the intermediate colonies perhaps corresponding to the Zwischenformen of the early German writers Since then Robinson (1934) has furnished the only description of secondary growth in young cultures, although Crowell (1926) studied dissociative phenomena in *C diphtheriæ* obtained from single cell cultures, unfortunately he gave little record of differential colonial features

Robinson described an *intermedius* strain of *C diphtheriæ* producing, among the normal colonies, others larger and atypical upon which papillæ developed in a few days and yielded unmistakable *gravis* colonies on subculture These colonies, however, failed to ferment starch until they had been trained for 3½ months by subculture in starch containing media

Stuart (1938) also isolated strains initially of *intermedius* colony form, which never produced a pellicle in broth culture, but which fermented starch and were serologically identical with *gravis* sub type B (Orr Ewing, 1933), though they produced no daughter colonies

ORIGINAL OBSERVATIONS

The strains of *C diphtheriæ* here described were discovered in the routine examination of about 1000 unselected positive throat cultures over a period of nine months About 14 in all have been observed,

which, while differing from Robinson's strain, are apparently examples of a similar dissociative process, probably in a later stage. They were all obtained from children under 16, most of whom were under school age and only three of whom had been immunised. All suffered mild or moderate illness and recovered without complications except that one or two had mild cardiac irregularity. There were three strains from one family. Throat swabs were inoculated on to plates of unheated blood tellurite agar (Johnstone and Zinnemann, 1943) and examined after overnight incubation at 37° C. By this time colonies could usually be classified by their naked-eye appearance but they were subcultured in starch medium and broth for confirmation. During this procedure colonies which on the tellurite plates had been frankly *intermedius* in architecture (fig. 1) were found to have fermented starch. Re-examination of the plates after further incubation revealed that the typical *intermedius* colonies of the previous day had developed daughter colonies. These papillary outgrowths were usually present on a majority of the basic colonies, though in some strains they had to be closely looked for. Some were small, single or multiple, others larger, single, semi-glossy, black in colour and heaped dome-shaped on the grey friable mother colony, whose edge projected around (figs. 2-4). The degree of tellurite reduction was variable, the smaller secondaries being frequently grey in colour and darkening with further growth. Subcultures from the black domes or grey papillæ on to the same unheated blood-tellurite medium or on to McLeod's original heated blood-tellurite medium produced a profuse large-colonied growth unmistakably *gravis* in appearance, grey in colour and breaking under the touch of a wire. Some colonies were typical daisy heads. There were no *intermedius*-like colonies, but subculture of colonies on the original plate which retained their *intermedius*-like form and did not produce daughter colonies (there were always a few of these even after several days incubation) reproduced in 16 hours a good growth of similar colonies which a little later displayed daughter colonies as described.

Several strains examined showed, after about the fourth transplantation, a few colonies some four times as large as the other *intermedius*-like growths; these were much nearer *gravis* forms in appearance than any other colonies on the plates. There were about 5 per cent. of these, some apparently clear of the mother colonies, many of which showed daughter colonies; others overlapping or inextricably mingled with the mother growth. There were now three colonial types : (1) almost frank *gravis* colonies ; (2) persistent *intermedius*-like colonies ; and (3) *intermedius*-like colonies with daughter colonies—some large and dome-shaped, others small, single or multiple. The frank *gravis* colonies bred true and produced typical *gravis* subcultures (fig. 3) ; the persistent *intermedius*-like colonies and those with daughter colonies reproduced a mixed growth as before. After at least 30 transplants from these *intermedius*-like colonies the

MICROBIC DISSOCIATION IN *C. DIPHTHERIÆ*
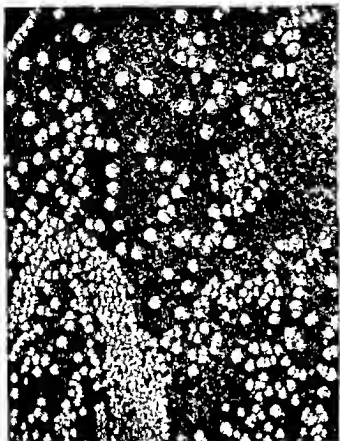
FIG. 1.—Colonies in initial *intermedius*-like phase growing on chocolate agar. Small white spots are early daughter colonies. ×4.
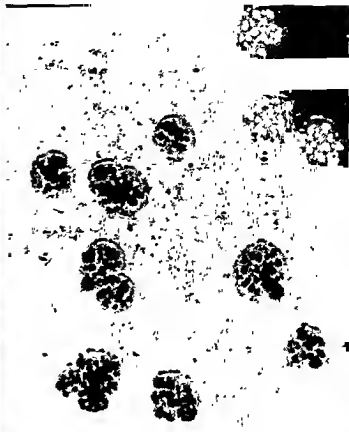
FIG. 2.—Growth on chocolate agar showing developed daughter colonies. Same strain as fig. 1. ×4.
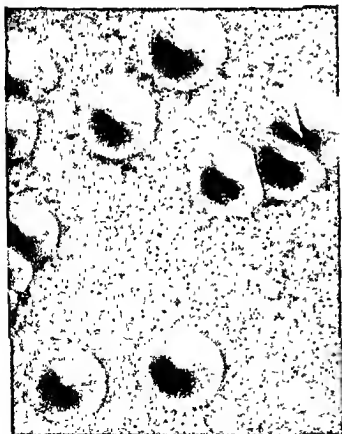
FIG. 3.—Colonies in *gravis* phase growing on heated blood tellurite agar. Same strain as figs. 1 and 2. ×6.

FIG. 4.—Colonies of another strain growing on unheated blood tellurite agar, showing *gravis* phase and daughter colony phase simultaneously developed. ×6.

Figs. 1 and 2 were produced in Professor J. W. McLeod's laboratory.

same process was still repeated. Even after many days' growth there were always a few persistent *intermedius*-like colonies which showed no detectable daughter colonies. Although several hundreds of such colonies have been tested, a non-starch-fermenter has not been found. All the strains fermented glucose and not sucrose. The growth in broth was at first like *intermedius*—a small, fine, granular deposit with clear fluid above ; but all cultures, especially if they were agitated, produced the pellicles typical of *gravis* strains.

All the observed strains displayed the characteristics described, but there were occasional variations during transition from *intermedius* to *gravis* colonial architecture. Sometimes fan-shaped outgrowths from the margins of the *intermedius*-like mother colonies were seen, grey and striated like *gravis* colonies, extending and enlarging until the original colony structure was overgrown ; or flat grey *intermedius*-like colonies studded with small secondary growths would spread laterally on the surface of the medium. Ultimately, when growth had apparently ceased, these presented the appearance of a thinned background suggestive of autolysis ; sometimes they showed apparent erosions and were studded with persistent small papillæ. Hadley (1927) gives no reference to erosive phenomena in *C. diphtheriæ*, but he claims to have seen plaque-like areas which he terms "invisible colonies" resembling areas produced by phage, and on them daughter colonies developing. Blair (1924) and d'Herelle (1926) mention a lytic principle connected with *C. diphtheriæ*. Very few of these "eroded" colonies were seen. In a few subcultures of some strains, after incubation up to 21 days, some original mother colonies with multiple daughter colonies would extend and modify until they ended as colonies typically *gravis* in appearance, striated and friable, with black centres formed of coalesced daughter colonies.

The persistent *intermedius*-like colony which as a rule got no larger on incubation had apparently no great longevity, for, after a few days, increasingly large inocula were required to obtain fresh growth. This seems to indicate that the primary mother colony was heading for destruction and individual organisms with *gravis* potentialities displayed the greater vitality.

Morphologically the bacilli in the primary *intermedius*-like colonies of 16-24 hours' growth on blood-tellurite media were essentially like those of true *intermedius* type but not so coarse. Longer forms predominated. Barring was the outstanding feature, with little tendency to clubbing. An occasional terminal granule could be seen. In the *gravis*-phase colony the organism displayed a rather florid *gravis*-type morphology with a good sprinkling of metachromatic granules. It was generally short but pleomorphic and showed very little barring. Between the two phases, mixtures blending form and other characteristics occurred, with distortions as the colonies aged.

All the strains isolated were virulent for guinea-pigs by sub-

cutaneous inoculation from Loeffler slope cultures. A suspension made from several persistent 'intermedius-like colonies from plate cultures was also virulent, but took longer to kill the animal. The first half-dozen strains were tested by agglutination of suspensions from Loeffler slope cultures made from colonies in the initial phase and fell into Robinson and Peeney's (1936) sub-type I, as the majority of Glasgow *gravis* strains do.

The cultural peculiarities of these strains suggested that starch fermentation might be due to the evolution of *gravis* dissociants from a basic true *intermedius* non-starch-fermenting type, so search for this was made.

Attempts to isolate a true intermedius *type*

Attempts to isolate an unmistakable *intermedius* type fulfilling all the criteria failed ; non-starch-fermenting colonies could not be isolated. The following methods were employed : selection of persistent *intermedius*-like colonies ; subculture on starch-containing media with the object of finding a non-starch-fermenting colony ; subculture on the medium of Gordon and Higginbottom (1942) (which was found unsuitable for single colony selection by colour reaction, though massed growth of these strains showed the greenish tinge characteristic of *C. diphtheriæ intermedius* in early growth but lost it on further development) ; subculture from ageing cultures in broth, on chocolate agar and on Loeffler's medium (old cultures generally returned *gravis* forms only, the heteromorphic organisms having died out) ; growth in the presence of antibacterial and antitoxic sera ; subculture from heated cultures in the manner of Mair (1936) ; animal passage (where recoveries, successful in every case, gave the organism still in its dissociating phase).

In addition, cultures from the first dissociating strain isolated were sent to Prof. J. W. McLeod, who very kindly examined them. The general findings were confirmed in his laboratory, but efforts by Dr Morris Gordon to induce a pure *intermedius* variant were unsuccessful, as were attempts by Dr K. I. Johnstone to obtain a single-cell culture for experimental purposes. Such cultures would, of course, have given a final answer to the possible argument that these are instances of an obstinate mixture of two forms of bacteria. Such phenomena have undoubtedly been observed, but in view of the invariably unsuccessful results of careful efforts at separation, this does not seem a likely explanation. The failure to obtain single-cell cultures by Johnstone's (1943) modification of Ørskov's method depends on the great difficulty of getting certain bacteria to grow from single cells when isolated on the surface of a solid medium, a fact which has not perhaps been sufficiently appreciated.

Cause of dissociative phenomena

Colony variants of *C. diphtheriæ* have often been described, especially in America, and anyone who has done much work on the bacteriology of diphtheria must have seen many. Most of these arise in laboratory cultures and are probably environmental and random variations. Those which occur in newly isolated cultures from persons suffering from diphtheria or from carriers, whether the variants are fully formed and colonially differentiated or display signs of being in a transitional stage, may perhaps be accepted simply as varying within the life phases of an enormously prolific community of individuals, where the chances of mutation and variation must be many.

Unfavourable environment has been adduced as one reason for dissociation and artificial methods have been used experimentally to produce variants of many bacteria. By analogy it is possible to consider mass immunisation as a biological experiment calculated to produce conditions in the human host unfavourable to the normal sequence of reproduction of the commonly recognised types of *C. diphtheriæ*, and also to produce in the strains circulating among the community modifications which occasionally show themselves in freshly isolated cultures by changes in colony structure, either completely achieved or in progress. Antitoxin production, which is dominant both in natural and artificial immunisation, is likely to obscure any response to minor somatic antigens of diphtheria bacilli coincidently present. But after mass immunisation the immunological mechanisms of a large number of people are relieved of this task and are therefore more likely to respond to such minor antigens introduced by the normal dissemination of diphtheria bacilli. Antibodies thus produced could be expected to have an influence on the structure or metabolism of the bacilli, and therefore, here and there, at one time or another, a situation may be created in which some strains of *C. diphtheriæ* fail to maintain recognised type individuality. The population at risk in Glasgow is roughly two-thirds immunised, and immunisation has been continuously pursued on a large scale during the last four years. This then may have created herd conditions unfavourable to the normal propagation of the types of *C. diphtheriæ* chiefly circulating in the community (*gravis* and *intermedius*), and thrown into the recognisable form of colonial modification some aberrations in their reproductive process. In bacteria showing dissociative phenomena, processes of reproduction differing from those usually accepted as normal have been suggested by German writers and elaborated by Hadley (1927, 1937). However this may be, the cultural abnormalities described above may, it is suggested, be accepted as evidence of an effort on the part of the micro-organism to stabilise a regressing morphologically stable type. It is unlikely to be a meaningless variation without significance in the life of the species.

Possible significance of variants of C. diphtheriæ *and dissociative change*

Although the so-called *mitis*, *intermedius* and *gravis* types of *C. diphtheriæ* have never been authentically transmuted artificially in the laboratory, they may be changeable in the hosts they infect, although it is only very rarely that we catch indications of such a possibility.

The dissociating strains described—few relative to the number isolated—are occurring at a time when some reduction of *gravis* predominance is taking place and coincident with a reappearance of atypical variants, chiefly types IV and VI, of which there have been very few for some years.

In the years before 1938 *intermedius* strains were predominant in Glasgow, rising to around 70 per cent. of all strains. Since that time the *gravis* type has rapidly assumed the ascendant, the *mitis* type remaining at a fairly constant low level as it has done ever since 1933. The percentage of *gravis* strains from over 6000 strains isolated during 1941-43 was 63·6 ; in 1944, 55·4 ; and in 1945, 49·5 ; while *intermedius* strains have increased by 8 per cent. during the past two years. In the years 1941-44 the percentage of atypical strains was only 0·46 in over 5000 strains ; in 1945 it was 4·9 in over 1400 strains.

During the rapid change over in 1937-39 from *intermedius* to *gravis* predominance alluded to above, the percentage was 6 in 3700 strains (Carter, 1943-44). Dissociation phenomena were not observed at that time, during the change in the chief infective type, perhaps because the process was so rapid. The present change, if it proceeds, is evidently much slower, which may account for the detection of heteromorphic organisms hovering between the *intermedius* and *gravis* isomorphs.

SUMMARY

1. Strains of *C. diphtheriæ* showing microbic dissociation by the production of daughter colonies are described.

2. Attempts have been made without success to isolate true *intermedius* types from these strains.

3. I have suggested how mass immunisation may be responsible for these dissociative phenomena.

4. Evidence from Glasgow shows that dissociation is perhaps most likely when type incidence is changing. I have suggested that it may represent an effort on the part of the micro-organism to stabilise a regressive type.

Comment by Professor J. W. McLeod

Dr H. S. Carter has expressed the wish that I should write a brief commentary on the findings recorded above. Their interest is obvious and they emphasise what should now be generally recognised—

namely, that diphtheria is not necessarily the same in all places at one time. It is from failure to realise or admit this fact that the mistake has been made of assuming that a faulty sample of prophylactic or the inoculation of too small a proportion of children are the only reasons why the results of diphtheria prophylaxis are inferior in this country to the most brilliant of those recorded elsewhere—in Toronto, for example.

Although my colleagues and I have handled large numbers of diphtheria strains of all types and from many areas in this country and beyond, we have not yet met the striking phenomenon so clearly shown in fig. 2, which was obtained with the strain sent to Leeds by Dr Carter.

The sequence of predominant *intermedius* infection followed by predominant *gravis* infection, which in some cases has approached epidemic proportions, has now been clearly recorded in Manchester by Robinson, in Liverpool by Wright, in Dundee by Tulloch, and in Glasgow by Carter, and the question arises whether these represent stages in the evolution of the diphtheria bacillus to its most active form. If this were so, however, one would expect that the dissociating form which Carter describes would have appeared in the period of increasing rather than of decreasing *gravis* incidence in Glasgow.

Another possible explanation of his observations, although a highly hypothetical one, is suggested by the green colouration on heated blood agar shown by these forms in their first phase, a colouration which is particularly well brought out by Dr Morris Gordon's expedient of adding unheated serum to heated blood agar plates. It is possible that the metabolic peculiarity of *intermedius* forms which leads to this oxidative effect on hæmatin is also responsible for the very restricted growth of this type on agar and heated blood agar media. If this is correct, then the phenomenon observed might well be one of an unusual *gravis* strain checked in its growth because it shared a metabolic peculiarity of the *intermedius* strain and returning to the normal *gravis* form as it shed this metabolic peculiarity.

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favour of the allergic theory. The lungs also may react, and in that case a characteristic form of pneumonia makes its appearance. Rheumatic pneumonia, which is very little known, is of great interest, as it has the characters of a transient lung infiltration and is therefore likely to throw light on this much discussed symptom. Recently Rich and Gregory (1943) have shown that in cases of rheumatic pneumonia changes are met with resembling those appearing in the lungs in connection with hypersensitiveness to sulpha- preparations. With the lung changes may be included rheumatic pleuritis.

Against the allergic theory it has been adduced that, contrary to expectation, glomerulonephritis and polyarthritis rheumatica are seldom met with in combination, glomerulonephritis also being considered an antigen-antibody reaction, with streptococcal products as the antigen. More detailed investigations by Salvesen (1938), Ehrström (1941) etc. have shown, however, that acute glomerulonephritis is more usual in cases of rheumatic polyarthritis than has been believed. Further, Klinge (1933) and Craciun et al. (1933) have found certain morbid anatomical changes in the kidneys in cases of polyarthritis without glomerulonephritis. Such cases are characterised by Ehrström as clinically dumb. Finally, in a certain percentage of cases of glomerulonephritis changes are met with in the larger vessels —not only in the kidneys but also in other organs—which resemble those in rheumatism and periarteritis nodosa (Fishberg, 1927 ; Masugi and Isibasi, 1935-36). This conforms well with the circumstance that glomerulonephritis is a frequent complication of manifest periarteritis nodosa, a fact which could perhaps also be expressed by saying that in certain cases of glomerulonephritis the general vascular changes are so severe that the disease might be designated periarteritis nodosa. Thus points of connection between polyarthritis and glomerulonephritis are not lacking.

Many attempts have been made to support the allergic theory by experiment, and it may now be said to have been established that changes can be called forth which are identical with those in polyarthritis and periarteritis nodosa by sensitising and reinjecting animals with foreign protein. On the other hand, little success attended attempts to provoke such changes with streptococci as antigen.

· Another method which can be employed is to investigate cases of allergy in man and compare the changes encountered with those characteristic of polyarthritis rheumatica. The object of this communication is to make just such a comparison. My material consists of four cases, all of which exhibited such severe allergic symptoms that death finally supervened. Slight joint symptoms were present in only one case (the other three were entirely free), and in none was there endocarditis. All had severe symptoms of asthma and were diagnosed clinically as such. Three exhibited transient lung infiltration and blood eosinophilia. Among other allergic symptoms present may be mentioned urticaria, nasal polypi, abdominal

THE ALLERGIC SYNDROMES

Fig 1—Case II Longitudinal section of a renal artery showing a bulge on one side, where the wall consists of a red stained mass formed by the completely destroyed media and adventitia The intima here is strongly proliferated its newly formed tissue partly filling the bulge Below the bulge, too, the intima is thickened There is no thickening of the intima in the opposite wall Mallory ×40

Fig 2—Case IV Transverse section of an artery in the lung In part of the circumference the wall is stained red owing to fibrinoid degeneration and necrosis The intimal proliferation is inappreciable here The wall and its surroundings are infiltrated with leucocytes including numerous eosinophils Mallory ×140

Fig 3—Case III Transverse section of an artery in the liver The intimal cells are much swollen and sometimes transformed into giant cells The greater part of the lumen is filled with proliferating endothelial cells eosinophils and other inflammatory cells These are also abundant in the vessel wall and its surroundings Mallory ×120

pain with diarrhœa and tenesmus, and eosinophilic pleurisy. As is usually the case in allergic diseases, the symptoms alternated. Microscopic examination showed vascular changes as in periarteritis nodosa, and such changes in the connective tissue in several organs as are characteristic of rheumatism or of the allergic inflammatory reaction generally. It is therefore not easy to say how the cases are to be classified, which shows that at times it is impossible to delimit the different allergic syndromes from each other, owing to the fact that the antigen-antibody reaction may affect many organs at the same time. Classification must thus be more or less arbitrary.

The vascular changes were focal and often localised to a part only of the circumference of the vessel. Both arteries and veins in different organs were attacked, but never the larger vessels. The lesions were of very varying ages in different vessels in the same case. Acute degenerative changes appeared in the form of disintegration foci in the elastica interna and tunica media, with fibrin reaction in hyaline necrotic masses (fig. 1, case II, and fig. 2, case IV). Sometimes in such places the wall was bulged out aneurysmally (fig. 1, case II). These acute degenerative changes were accompanied by a cellular exudation in and around the vessel wall (fig. 2, case IV, and fig. 3, case III). Among these cells were numerous eosinophils. Proliferative changes also occurred, first in the intima, the endothelial cells of which often assumed the character of giant cells (fig. 3). In the smaller vessels these cellular proliferations might fill up the lumen, leaving only quite small spaces in which occasional red blood corpuscles were observed (fig. 4, case IV). In the more advanced lesions the proliferating cells formed collagen fibrils, so that finally a loose connective tissue resulted. This might appear only as an excrescence in the lumen or might fill up the whole lumen, including even an aneurysm or a defect in the wall (fig. 1). At this stage the cellular exudate had completely receded.

In addition to these vascular changes, which in themselves are in no way remarkable, there were changes also in the connective tissue of several organs of the kind found in cases of polyarthritis rheumatica, both in the form of so-called rheumatic "Frühinfiltrat" and as rheumatic granulomata. As is well known, the rheumatic "Frühinfiltrat" is characterised by œdema and by the collagen fibres being focally swollen and split up into fine fibrils which stain black with silver, in contrast to connective tissue fibres, which become brown or dull purple. Sometimes, but not always, these fibres assume a positive fibrin colour, and the changes are therefore called "fibrinoid degeneration". Such changes are met with especially in the interlobular septa of the lungs and in the adventitia of certain vessels, but also in other places (figs. 5 and 6, case II).

Rheumatic granulomas are also met with, chiefly in the lungs. In principle they exhibit the same changes in the connective tissue as the "Frühinfiltrat", but in addition there is an accumulation of

large basophilic cells with abundant protoplasm between the fibrils
or in the periphery of the focus. These cells resemble macrophages
but are in fact fibroblasts. They may be multinuclear and transformed
into giant cells (fig. 7, case I). The collagen fibrils in the centre of the
granuloma often disintegrate (fig. 8, case I). Sometimes disintegration
of the collagenous fibres and the formation of giant cells without
distinct granuloma formation are seen (fig. 9, case III.). As is well
known, the rheumatic granulomata were first observed by Aschoff
in the myocardium, and when so located are called Aschoff's nodules.
In my series granulomas of this kind were present in the myocardium
in one case only (fig. 10, case I). It should be pointed out that this
patient had not suffered from polyarthritis or endocarditis and did
not exhibit any changes in the joint capsules or endocardium.
Rheumatic granulomas may fuse to form conglomerate granulomas,
which in some cases of chronic polyarthritis cause nodes in the skin
around the joints. Similar pictures are also seen in the cutaneous
nodules occurring in the neighbourhood of tendon sheaths and joints
which characterise the skin disease called granuloma annulare. Such
conglomerate granulomas were present in my cases in the interlobular
septa of the lungs (fig. 8, case I).

Apart from the vascular changes, rheumatic " Frühinfiltraten "
and the granulomas just described, the changes in the lungs were of
very special interest in view of the fact that during their lifetime
the patients had exhibited transient lung infiltration, with blood
eosinophilia.

The concept of transient lung infiltration was introduced in 1931
by Löffler. This syndrome is characterised by a pronounced blood
eosinophilia with values up to 70 per cent, or more, and X-ray shadows
in the lungs which are variable in structure, solitary or multiple,
unilateral or bilateral. They are formed rapidly and may disappear
in 3-8 days, suddenly to reappear at the same place or elsewhere in
the lungs. In spite of these large shadows, the physical symptoms
are comparatively slight, and in the majority of cases the patients
exhibit no very grave symptoms. At times the infiltration is met
with in apparently healthy persons who have been subjected to
routine X-ray examination. In general, however, the patients suffer
from fatigue, darting pains in the chest, and an irritating cough,
with yellow, slimy sputum poor in cells. It was not long, however,
before it became clear that this infiltration appeared especially in
persons who suffered from allergic diseases such as hay fever, angio-
neurotic œdema, vasomotor rhinitis, urticaria, migraine, pseudo-
appendicitic irritation and asthma. Of Maier's (1943) 100 cases 52
had other allergic symptoms.

During the course of years the original conception of the Löffler
syndrome has changed in character. At first it only comprised cases
of acute illness, but gradually cases have been included in which the
morbid histories were very long, and where lung infiltration combined

THE ALLERGIC SYNDROMES
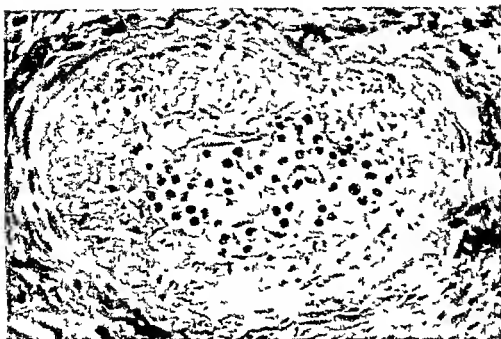
Fig 4—Case IV Section of a small vein in the lung The lumen is completely blocked by large proliferating intimal cells with abundant protoplasm these have formed a few small fibrils In the interstices there are occasional red blood corpuscles Mallory ×270

Fig 5—Case II Section of lung showing connective tissue with fibrinoid degeneration (red stain) in an interlobular septum which is very much increased in breadth Mallory ×350
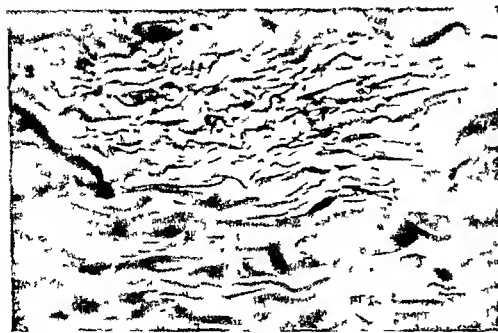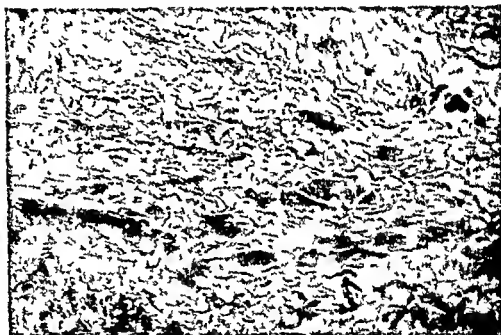
Fig 6—Case II Small silver stained focus of fibrinoid degeneration in the adventitia of a renal artery The focus is surrounded by connective tissue fibres which stain dull purple In the focus itself the connective tissue fibrils are split up into fine black fibrils With van Gieson staining the focus becomes yellow with Mallory red streaks are seen in it ×900

with blood eosinophilia came and went for months and years, or persisted unchanged for long periods. In some of these cases the course of the disease was mild; others diverged from the ordinary Löffler syndrome in that the course was malignant.

The morbid anatomical basis of this transient lung infiltration has been the subject of much discussion. A number of workers have regarded the lesion as an allergic lung œdema, a parallel phenomenon to Quincke's œdema of the skin, and have stressed its similarity to Osler's visceral angioneurotic œdema, which is characterised by pleomorphic skin affections such as erythema, urticaria, Quincke's œdema, purpura etc., and visceral symptoms in the form of headache and other cerebral symptoms, pain in the abdomen with rigidity, vomiting and melæna, and in a number of cases also by transient lung symptoms. Various authors (Vaughan and Hawke, 1930-31; Cole and Korns, 1933-34; Miller *et al.*, 1935; etc.) have published cases of transient lung infiltration which are best explained in this way. Vaughan and Hawke's case was that of a young woman who suffered from hypersensitiveness to tomatoes and eggs, and who exhibited the following collection of allergic symptoms, appearing on different occasions, namely abdominal pain, vasomotor rhinitis, transient X-ray shadows in the lungs, transient hydrarthroses, skin affections, paræsthesiæ, bilateral ulnar paralysis, vertigo, partial blindness and nystagmus, but not asthma. The allergic symptoms were provoked in this case by a non-bacterial antigen.

Very similar are some of the cases described by Söderling (1939) and those published by Engel (1935-36) from Shanghai under the name of " privet cough ". The latter, which appeared regularly during May and June and is characterised by coughs, colds in the head, facial œdema, transient lung infiltration and blood eosinophilia, is considered by Engel to be due to hypersensitiveness to the pollen of Ligustrum.

The few cases of transient eosinophilic lung infiltration which have come to autopsy, however, appear to show that, even in the slightest cases, the changes are not restricted to œdema. In 1942 von Meyenburg published four such cases. These had not been examined clinically in respect of the lungs, but the lung lesion was of such a nature that he considered it to be transient eosinophilic lung infiltration. Three were soldiers on active service who died suddenly as a result of accidents. One had suffered from asthma in childhood. In the fourth case death was due to tetanus. The lungs exhibited inflammatory changes which diverged distinctly from the ordinary, in respect of absence of fibrin, high content of eosinophils, and a pronounced affection of the interstitial tissues. A remarkable fact was the formation of giant cells in the alveoli and in one case of granulomas, which, judging from the description, must have been very much like the rheumatic granuloma. Finally, it should be pointed out that in one of the cases, in spite of its acute and completely

benign character, von Meyenburg found small "thrombophlebites"[1] in the interstitial lung tissues. The changes were not confined to the lungs, eosinophils being also met with in the interstitial tissue of the liver. This has since been confirmed by Maier, who mentions cases with eosinophilic infiltration of the peripheral lymph glands, epididymis and musculature, and draws the conclusion that in cases of eosinophilic lung infiltration there is an allergic reaction involving the whole body.

In all my cases the disease is of a more chronic character, and, as might be expected, the changes are somewhat different from those in von Meyenburg's cases. They exhibit, however, a striking resemblance in principle. Macroscopically the picture is dominated by an increase in breadth of the interlobular septa, which, in the form of white streaks, chequer the cut surface of the lungs. Microscopical examination proves this increase in breadth to be largely conditioned by a new formation of connective tissue. The previously described rheumatic "Frühinfiltraten", with granulomas and vascular changes, are observed in this connective tissue, which also is more or less infiltrated by plasma cells and eosinophils. The bronchi exhibit the usual changes of asthma.

The interstitial pneumonia observed by von Meyenburg is thus found in a chronic form in my cases. The lung parenchyma itself exhibits different changes in different areas. In some areas only thickening of the alveolar walls with great distention of their capillaries is seen, accompanied by swelling of the alveolar epithelium, and a serous exudate and occasional eosinophils in the alveoli. Elsewhere proliferative processes have made their appearance. The alveoli are filled with large polymorphous cells (fig. 11, case III), some of which have the character of giant cells. In all probability it is not a question here of alveolar epithelium but of fibroblasts. In every case connective tissue is gradually formed in the alveoli, giving rise to indurated areas (fig. 12, case II). Here and there the connective tissue proliferates into the small bronchi. In certain areas the alveoli are filled with fat phagocytes, and in many places around the latter there is connective tissue fibril formation.

The interstitial inflammation, with serous exudate and eosinophil cells, and giant cells in the alveoli, is common to von Meyenburg's cases and my own. In mine, however, pronounced proliferative processes with fresh formations of connective tissue had appeared, owing to their chronic character.

The changes now described appear, however, to have a still greater resemblance to those met with in rheumatic pneumonia, the pathological anatomy of which has been described in recent years by Naish (1928), Gouley and Eiman (1932), Rich and Gregory (1943) etc. As already mentioned, these pneumonias have the character of transient lung infiltration.

Pathologically the rheumatic lung is characterised by an interstitial pneumonia with thickening of interacinous and interlobular

THE ALLERGIC SYNDROMES
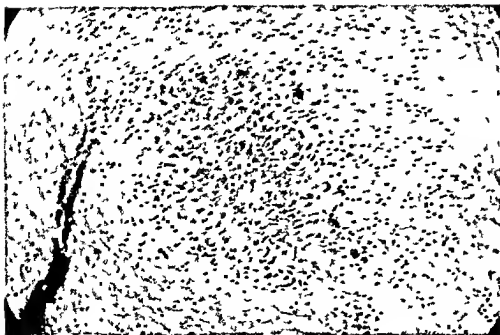
Fig 7 —Case I   Granu loma in an interlobular septum of the lung   The granuloma consists of swollen fibroblasts, some of which have assumed the character of giant cells, and fine fibrils, seen only in the periphery of the granuloma   In the centre, disintegration has taken place   Around and even within the granu loma there are lympho cytes   and   occasional eosinophils   van Gieson × 140

Fig 8 —Case I   Con glomerate granuloma in an interlobular septum of the lung, consisting of four small granulo matous foci   The black staining in the centre of each granuloma is caused by disintegration of the fibrils   Silver staining × 80
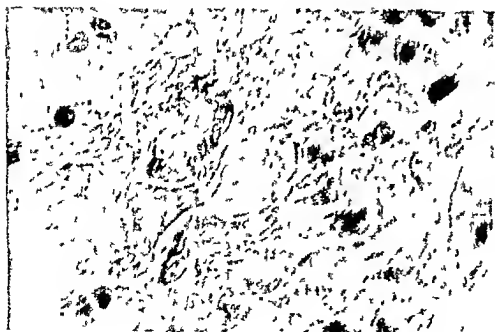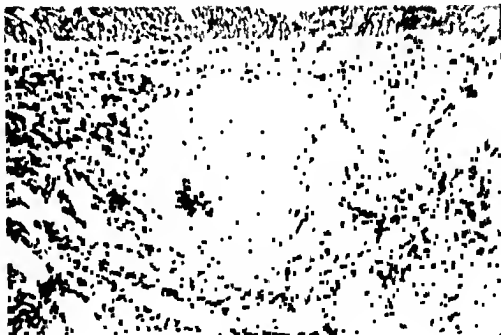
Fig 9 —Case III   Section from the pericardium showing disintegration of collagenous fibres and pro liferating connective tissue cells, some of which have assumed the form of giant cells   Only fragments of the collagenous fibres (red stained) are visible   van Gieson   × 700

THE ALLERGIC SYNDROMES
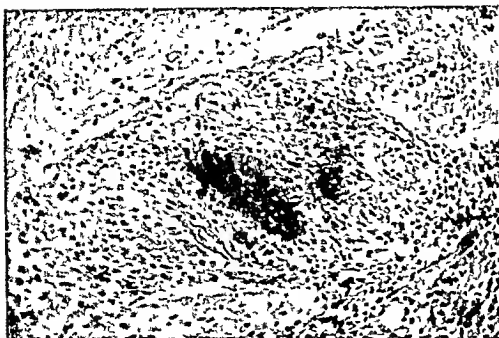
Fig. 10 —Case I Section from the myocardium showing a nodule composed of large swollen fibroblasts and disintegrated connective tissue fibres, surrounded by a fairly abundant cellular exudate Some of the muscle fibres at the margin are destroyed van Gieson × 150

Fig 11 —Case III Lung alveolus containing mononuclear polymorphous cells, some with processes and giant cells To the right can be clearly discerned a row of swollen alveolar epithelial cells delimiting the alveolar space van Gieson × 750
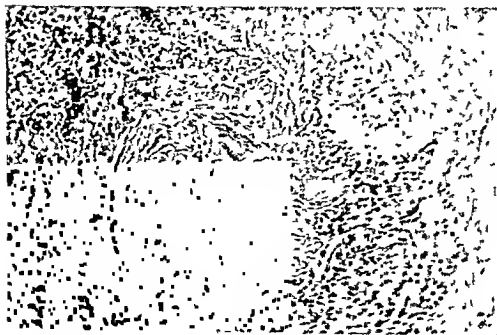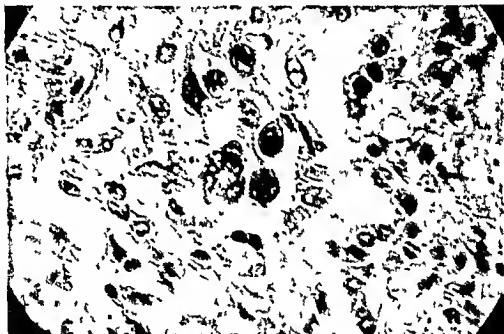
Fig 12 —Case II Portion of lung showing alveoli filled with newly formed connective tissue and giant cells Mallory. × 250

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septa, which, macroscopically, stand out as a greyish-white network on the dark red background of the cut surface exactly as in my cases. Microscopic examination of these septa reveals œdema and infiltration by basophilic cells with abundant protoplasm, giant cells and lympho-cytes, but only occasional polymorphonuclear leucocytes. Granulomas with and without central necrosis are also observed which exactly resemble Aschoff's nodes. The alveolar walls are thickened owing to congestion, œdema and cellular infiltration. On the other hand, in the alveoli themselves only slight exudate is seen or none at all, but swollen alveolar epithelium and proliferating cells are present. Rich and Gregory found also capillary thromboses and focal necroses in cases of rheumatic pneumonia, and in one case Gouley and Eiman observed vascular changes resembling those of periarteritis nodosa.

If all these findings are collated, it appears that the conclusion can be drawn that this transient lung infiltration is due to an allergic inflammation of the lungs, which may be both acute and chronic in character. A parallel must then be drawn between the transient nature of the infiltration and the polyarthritic joint and skin changes. The disappearance of often considerable rheumatic nodes in the skin may take place in 1 or 2 days, which is explained by the fact that the acute changes may be confined to serous exudation and swelling of the connective tissue fibrils. If proliferative processes with the formation of granulomas appear, the nodes may remain for months and years. Conditions are obviously the same in the lungs.

So far as can be judged, transient lung infiltration, like asthma, may be evoked by antigens of various kinds. In certain cases a bacterial antigen must be taken into account, as in some cases of asthma. As is well known, a distinction is now made between a slighter " extrinsic " form of asthma and a severer " intrinsic " form which is often fatal. In contradistinction to the former, the intrinsic form is considered to be caused by a bacterial antigen. The state of affairs is probably similar in transient lung infiltration, and the cases published here, with their grave changes, would then be of the more severe form. Certain statements in the literature indicate that this analogy may perhaps be extended to allergic diseases in general, e.g. Ehrström's cases of glomerulonephritis caused by non-bacterial antigens.

Cases such as those now reported are certainly not unusual. If the literature is gone through, a number of cases of manifestly similar nature are found published under different headings (Rössle, 1933 ; Meyer-Dörken, 1934 ; Harkavy, 1941 ; Alwall, 1943 ; Bayley et al., 1945 etc.). In general, however, apart from other allergic phenomena, joint symptoms have usually been present. Special interest attaches to Harkavy's 8 cases, which exhibited an unusually large number of allergic symptoms.

Case reports

Case I. A 43-year-old woman had suffered for nearly 15 years from vomiting and abdominal pain, and for 7 years from allergic symptoms affecting the upper respiratory passages. For 2 years she had had severe asthma, and towards the end these symptoms had developed into constant difficulty in breathing, while at the same time the abdominal symptoms progressed to severe diarrhœa, leading to cachexia and death. Before this, bilateral pleurisy had supervened. A high blood eosinophilia and transient lung infiltration also formed part of the morbid picture.

At autopsy the lungs exhibited a great increase in breadth of the interlobular septa. In these septa were seen rheumatic "Frühinfiltraten" and numerous rheumatic granulomas. The latter were also observed in the myocardium, pericardium and pleuræ. The lung parenchyma itself was the seat of serous exudation, eosinophilia, fat macrophage accumulation, fibroblastic proliferation and giant cell formation in the alveoli, but without the formation of fibrinous exudate. The lungs also exhibited the changes characteristic of bronchial asthma. Finally, in the lungs and liver a periarteritis nodosa in different stages of development was observed.

Case II. A 17-year-old youth with an allergic heredity was healthy until the age of 14, when he began to have repeated attacks of rhinitis. He also became short of breath and had slimy sputum, which was sometimes bloodstained. At the age of 16 he developed manifest asthma, with transient lung infiltration and blood eosinophilia. Gradually pleurisy set in and also abdominal symptoms, and he presented the picture of general marasmus. During the period immediately before death he had diarrhœa but no attacks of asthma.

At autopsy the lungs exhibited the same increase in breadth of the interlobular septa as in case I. In these septa were observed fibrinoid degeneration of the connective tissue and rheumatic granulomas. The lung parenchyma exhibited collapsed areas, and in these the alveoli contained the same characteristic cells as in case I. The collapsed areas were in process of being transformed into connective tissue. In some bronchi the wall was broken through and the newly formed connective tissue had proliferated into the lumen. These changes explained the occasional occurrence of blood-stained sputum. The bronchi contained no mucus, and there were no goblet cells. Thus the picture diverges from what is seen in persons dying in an acute asthmatic attack, which is probably due to the fact that towards the end of his illness the patient had no such attacks. Fully developed rheumatic granulomas were not met with in any organ except the lungs, but connective tissue changes resembling those in the rheumatic "Frühinfiltrat" were observed in the pericardium, capsule of the spleen, tongue and adventitia of certain arteries. Periarteritis nodosa was observed in slides from different organs. Numerous streptococci were present in nearly all organs.

Case III. A woman who, during childhood, had had scarlatina with albuminuria, fell ill at the age of 29 with polyarthritis but recovered. At 37 she developed sinusitis and asthma and a year later she suffered from erysipelas, with subsequent blood eosinophilia and transient lung infiltration. Within a short time she developed swelling and pain in a number of small joints, her general condition deteriorated, and she died about 1½ months after falling ill.

At autopsy the lungs exhibited the bronchial changes characteristic of asthma, with, in addition, the changes in the interlobular septa and alveoli which characterised cases I and II, but in a less pronounced form. There were very extensive periarteritic changes in many organs, and the myocardium showed great damage as a result of periarteritis nodosa of the coronary arteries. Granulomas were observed only in the peri- and myocardium. Many of the lymphatic glands of the body were heavily infiltrated with eosinophil cells.

Case IV. A previously healthy 31-year-old woman fell ill with cough and wheezing in the chest, and finally developed attacks of asthma. She died of cardiac weakness after only 2¼ months' illness. Towards the end urticaria developed, and the sputum contained bright red blood. Histological examination was confined to the lungs. In addition to the asthmatic lesion they showed diffuse periarteritis nodosa with almost exclusively acute changes. The interlobular septa were greatly thickened, as in cases I-III, but the changes were limited to œdema and great cellular infiltration, especially with plasma cells and eosinophils. The alveoli exhibited emphysema, hæmorrhage and a cellular exudation but no formation of connective tissue. Case IV thus appears to show an early stage of the same morbid picture as cases I-III.

Summary

Four cases are reported which ended fatally with a morbid picture characterised by a variety of allergic symptoms, such as asthma, transient lung infiltration with blood eosinophilia, nasal polypi and abdominal pain with diarrhœa. Of these symptoms asthma was the most conspicuous and in consequence the cases were diagnosed as asthma. Histological examination revealed—apart from the ordinary asthmatic lesions—such vascular changes, both in the lungs and in various other organs, as are characteristic of periarteritis nodosa and polyarthritis rheumatica. Further, in the lungs and other organs there were observed foci where the connective tissue exhibited fibrinoid degeneration—the so-called " Frühinfiltrat "—and rheumatic (including conglomerate) granulomas. In spite of these characteristic " rheumatic " changes the patients had not suffered from endocarditis or polyarthritis, with the exception of one who had had slight joint trouble. The lung changes exhibited close agreement with those described by von Meyenburg as characteristic of acute transient lung infiltration, namely interstitial inflammation and serous exudation, with eosinophils and giant cells in the alveoli. In the author's cases, however, there were also proliferative processes, indicating the chronic character of the lesion. Nevertheless the lung changes exhibited a still greater resemblance to those described in cases of so-called rheumatic pneumonia. It is concluded that the clinical picture of so-called transient lung infiltration with blood eosinophilia corresponds to an allergic inflammation in the lungs, which may follow either an acute or a chronic course. The more or less transient nature of the infiltration in many cases is analogous to the transience of the rheumatic swelling of joints and the rheumatic nodes in the skin, which may disappear in about 24 hours or may remain for months or years.

The author regards such syndromes as polyarthritis rheumatica, periarteritis nodosa, endo-, myo- and pericarditis rheumatica and transient lung infiltration with eosinophilia, etc., as equivalents, i.e. manifestations of the antigen-antibody reaction localised to different organs. The clinical classification of cases is dependent on the particular organ in which the reaction is most pronounced, but cases are met with where so many organs react at the same time that a topographical

classification is almost impossible. As regards the transient lung infiltration, it would appear that, as in the case of asthma, a distinction can be made between a slighter " extrinsic " form, where the antigen is non-bacterial, and a severer " intrinsic " form, where it is bacterial. Certain statements in the literature indicate that this analogy might perhaps be extended to include the allergic syndromes in general.

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PATHOLOGICAL CHANGES INDUCED BY LEWISITE AND ALLIED COMPOUNDS

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From the Experimental Station, Porton

(PLATES LVIII AND LIX)

WE describe a group of poisons which produce constant and wide-spread effects in laboratory animals when administered by various routes. The toxicological action of the prototype of the group, 2 chlorovinyldichloroarsine (lewisite I), was recognised early in 1940 and most of the subsequent investigations were carried out with this compound. Information about some of the other members is less full, because only a small amount of material was at our disposal ; but there is good reason to believe that in general all behave in much the same way, and similarity of action after skin application means that closely related effects follow other routes of administration. For convenience we shall refer to these poisons as the lewisite group, despite differences in chemical constitution of individual members. The group includes lewisite I ($CHClCHAsCl_2$), lewisite II [$(CHClCH)_2AsCl$], phenyldichloroarsine, phenyldibromoarsine and phenyldifluoroarsine. All are fairly heavy liquids, with pungent and often characteristic odours. They are soluble in many organic solvents but tend to be changed in water.

Fatal doses. The LD_{50} for various routes are summarised in the table ; some figures for hydrolysed lewisite I (2 chlorovinylarsenjousoxide), usually referred to as lewisite oxide, are included. Undiluted compounds were used for skin and subcutaneous tests. Dilution, when necessary, was carried out with arachis oil or liquid paraffin : this was essential for intravenous injection. We also determined, for lewisite I, the smallest skin dose producing serious, though not fatal, changes. Adopting biliary lesions as the measure of casualty production in guinea pigs, the smallest effective dose appears to be one fifth to one-third the LD_{50}. In rabbits, one third the LD_{50} is the smallest dose capable of inducing definite blood changes.

PATHOLOGICAL CHANGES INDUCED BY THE LEWISITE GROUP

1. *Local effects on the skin*

A. Human. We performed biopsies on human subjects who had had small drops of pure lewisite applied to the skin. We also had access to similar material prepared by American colleagues. Our

specimens were fixed in Susa and stained by a large variety of methods. A blister is well in evidence 24 hours after the application of the lewisite.

TABLE

LD_{50} (mg./kg.) for the lewisite group of compounds

	Lewisite I	Lewisite I oxide	Lewisite II	Phenyldichloro-arsine	Phenyldibromo-arsine	Phenyldifluoro-arsine
Skin						
Goat . .	15	...	...	...	...	...
Dog . .	15	...	...	...	...	...
Rabbit .	6	...	...	3	4	4
Guinea-pig.	12	...	8	4	6	10
Rat . .	24	...	...	16	15	15
Subcutaneous						
Goat . .	...	...	...	...	...	...
Dog . .	2	...	...	...	...	...
Rabbit .	2	...	...	...	...	...
Guinea-pig.	1	0·2	0·2	...	...	...
Rat . .	1	...	...	...	...	...
Intravenous						
Goat . .	...	...	...	...	...	...
Dog . .	...	...	...	...	...	...
Rabbit .	0·5	1	...	0·5	0·5	0·5
Guinea-pig.	...	...	...	...	...	...
Rat . .	...	...	...	...	...	...
Oral						
Goat . .	...	...	...	...	...	...
Dog . .	...	...	...	...	...	...
Rabbit .	...	3	...	...	...	...
Guinea-pig.	...	2	...	...	...	...
Rat . .	...	5·15	...	...	...	...

The blister and epidermis. The line of separation in the formation of the blister is between the deepest layer of the rete malpighii (stratum germinativum) and the subjacent dermis. No vestiges of dermal reticulum, collagen or elastic fibres adhere to the roof, which is therefore composed of the whole thickness of the epidermis and of this only. At the centre of the blister there is considerable thinning of the epithelial layers. Except at the edges of the blister, epidermal cell structure is almost wholly lacking and stains a uniform pink with eosin, though the stratum corneum is still recognisable. Most of the nuclei are very degenerate, many having completely disappeared, while others are represented only by the nucleoli. At the extreme edge of attachment cells and nuclei show slightly more structure, though the cytoplasm may be hydropic. The blister fluid precipitates as a fine reticulum. Hair follicles and ducts of sweat glands may cross the blister, the ducts showing considerable damage, the hair follicles often less affected. Fine strands of fibrin are deposited around these structures and on the superficial layers of the dermis.

The dermis. Starting from the edges of the blister and curving

downwards through the deeper layers of the dermis is a more or less regular band of degenerate cells consisting of almost equal numbers of polymorph leucocytes and histiocytes Near the attachment of the blister roof, these cells are very numerous At the centre of the band they are more scattered Lying just below the floor of the blister there is often a narrow second band of scattered degenerate polymorphs This evidently represents the initial response in the surface layers of the dermis whilst absorption of lewisite is going on more deeply into the dermis Its restricted nature suggests that this first leucocyte response ceases, possibly as a result of circulatory stasis or because of unfavourable conditions

Fluid separates the collagen bundles of the dermis and here the fibrin deposit is prominent, forming a more deeply staining reticulum than within the blister

Throughout the dermis, capillary congestion is very marked and a high proportion of vessels show swelling of endothelial nuclei and, near the centre of the lesion, loss of structure of their walls, the result of coagulation necrosis A few contain thrombi Lymphatics are unduly prominent and a few contain thrombi

B Animal The most convenient place for the study of skin changes is the rabbit's ear Coagulation necrosis involving all types of tissue sets in rapidly and generally reaches its greatest extent by the third day A slough eventually forms and separates after two or three weeks Surrounding this necrotic area is a broad zone of œdema, with lymphatic dilatation and sometimes lymphatic thrombosis Blister formation is rare such blisters closely resemble those developing in human skin

Migration of leucocytes from all types of vessels can be demonstrated as early as one hour after the application of lewisite and reaches its maximum in 2 3 days At this time, concentrations of leucocytes form a complete investment for the necrotic area and adjacent capillaries are packed with polymorphs Histological evidence of increased capillary permeability is afforded by the great œdema which is present Capillary hæmorrhages at the edge of the necrotic area and in the media of arterioles are common

During the period of sloughing, intense proliferation of fibrous tissue takes place around the central necrotic area Hæmorrhages around the new capillaries point to fragility of their walls Fibrosis involves the nerve sheaths and individual nerve fibres may be surrounded by increased fibrous tissue Hæmorrhage within nerve trunks has not been demonstrated The neuritis is therefore due to the direct action of the lewisite

An interesting late effect in arteries has been constantly noted Between the third and sixth week degeneration of the muscle fibres of the media and their subsequent replacement by fibrous tissue occur In sections of normal rabbit's ears stained by van Gieson and orcein the muscle tissue of the media forms a well defined yellow

layer limited internally by the internal elastic lamella and externally by the condensation of elastic tissue at the medio-adventitial junction. This layer contains only a small proportion of fibrous tissue. In late lewisite lesions, however, the muscle cell nuclei become degenerate and shrunken, giving a vacuolated appearance to the media. At the same time there is a much higher proportion of fibrous tissue. There is constant endothelial and intimal thickening, due apparently to proliferation of endothelial cells and to a considerable deposit of fibrous tissue in the intima. The contrast between this thickened intima and the normal is very striking in some preparations. Defects in the internal elastic lamella have also been found at this stage.

Resistance to necrosis shown by cartilage is marked. Ultimately it too becomes involved and may be responsible for a secondary leucocyte emigration in the fourth week. After 4-6 weeks fibroblastic regeneration of cartilage can be seen. Hair follicles seem to possess some degree of resistance to lewisite : their essential structure and staining power are fairly well preserved on the second day after its application.

2. *Effects following injection*

Severe local acute inflammation with much necrosis and extensive œdema follows subcutaneous injection. Absorption of lewisite occurs rapidly and leads to systemic effects. Intramuscular injection gives much the same results. Intravenous injection of lewisite in arachis oil or of lewisite oxide in water may be followed by death if the dose is fairly large, or may give rise to systemic effects only. Thrombosis of the injected vessel is common. Similar results are produced by other members of the group.

3. *Effects of inhalation of lewisite I vapour or of droplets*

Because of the intensely irritating action of lewisite I, it is difficult to induce the animal to inhale sufficient of the compound to study its effects. Laryngeal spasm is severe and may cause death from asphyxia. Droplets produce the same sequel. But quite small concentrations can be inhaled and lead to serious effects if maintained over a prolonged period of time. Nasal irritation results in acute muco-purulent rhinitis, the accessory sinuses also being affected. Acute pharyngitis, laryngitis and tracheitis are seen within a few hours and fibrinous inflammation induces the formation of a false membrane covering the whole of the tracheal mucosa. Severe ulceration and œdema of the wall often develop. Acute fibrinous bronchitis is pronounced, and a cast may spread to the terminal bronchi, with resulting pulmonary collapse. Pulmonary œdema is common during the first 24 hours but soon gives place to acute bronchopneumonia. These lesions are indistinguishable from those so well known after

mustard gas inhalation. Pleural effusion is not uncommon and rarely empyema. Bronchial lymph glands become enlarged and hæmorrhagic. Systemic effects are rare.

4. *Effects following ingestion*

Aqueous solutions of lewisite oxide and alcoholic solutions of lewisite give prominent changes in the alimentary canal as well as systemic effects. Acute inflammation of the mucous membrane of the stomach or intestine is characterised by severe hæmorrhage, necrosis of epithelium and submucous œdema (fig. 4). Occasionally, a large dose may lead to perforation of the stomach wall. Much mucus is secreted and is usually mixed with necrotic debris to form a false membrane on the surface. When the stomach is full of food, lesions are mostly confined to that organ and pyloric spasm is pronounced, but if the poison be given on an empty stomach, the maximum damage is in the duodenum and upper jejunum. This is probably due to the rapid emptying of the stomach, which contains only fluid. If lewisite oxide be introduced directly into a rabbit's duodenum after laparotomy, an area of intense inflammation results at the point of injection and along the bowel for a few centimetres in both directions. Acute inflammation is also found in the stomach, apparently the result of regurgitation of duodenal contents. This can be proved to be the case by injecting lewisite oxide into the duodenum of a rabbit in which a ligature has been tied round the pylorus; in this animal the inflammation extends proximally only as far as the ligature; the stomach shows no abnormality. From these observations it seems clear that the distribution of the lesions is determined by the mechanics of the alimentary tract; the alkaline duodenal secretion appears to have no influence in modifying the action of the poison.

No evidence of damage to the alimentary tract has been found below the upper jejunum; frank blood or dark-coloured fæces may be found in the terminal ileum and colon, but the wall of these parts is not damaged. If lewisite be introduced directly into the gut, great destruction of the wall follows.

5. *Systemic effects*

These develop rapidly after skin contamination, ingestion and subcutaneous, intravenous or intraperitoneal injection. They are rare after inhalation.

Cardiovascular system. In the heart no characteristic changes are seen, macroscopically or microscopically, following the administration of small or large doses of lewisite by various routes. Animals dying within the first 24 hours often show acute dilatation of the heart cavities, especially the right. Pericardial effusion is infrequent,

pericardial and endocardial hæmorrhages are rare except in the goat, dog and cat. Sometimes small capillary and venous hæmorrhages develop in the myocardium but are inconstant in their situation. The cardiac muscle fibres seldom show any pathological change beyond slight cloudy swelling and fatty degeneration. The A-V bundle is not infrequently involved in subendocardial hæmorrhage in the goat.

Vascular damage is the rule and takes the form of scattered petechiæ in the serous membranes, especially the peritoneum ; rarely, large sub-peritoneal hæmorrhages are found overlying the lumbar muscles.

Respiratory system. Lesions following inhalation have already been described ; the following description applies to other routes of administration. Pulmonary lesions are uncommon in the guinea-pig and mouse, frequent in the rabbit and rat. Severe pulmonary changes develop rapidly after intravenous injection and include hæmorrhage, extensive œdema and compensatory emphysema. In animals which survive, patchy alveolitis and bronchiolitis are seen. Pleural effusion is common and often severe in the goat, dog and cat.

Biliary system. Striking changes are seen within a few hours in the bile passages and liver of the guinea-pig, rabbit, cat, dog and goat, but not in the rat and mouse. The guinea-pig is especially susceptible and is the ideal animal for the experimental study of lewisite poisoning.

Three hours after the skin application of lewisite to guinea-pigs the liver appears swollen and congested. The gall bladder and bile ducts, especially the common bile duct, are distended with watery bile, which may be blood-stained. The wall of the gall bladder may be opaque, with a few tiny hæmorrhages and congestion. The wall of the common bile duct is sometimes white and opaque or faintly tinged with red. This is especially prominent in the terminal 0·5 cm. of its course, where it enters the duodenum. Microscopical examination shows small areas of necrosis in the mucosa of the gall bladder, the lining epithelium being destroyed or desquamating, the underlying tissues congested and œdematous. Small and medium sized hæmorrhages occur in the mucosa and deeper tissues. The terminal part of the common bile duct shows desquamation and destruction of the lining epithelium, so that the wall is completely denuded and the lumen filled with debris and bile. Liver cells near portal canals may have lost their mitochondria in 2-3 hours. Small groups of cells in this area stain diffusely with intravital trypan blue, suggesting severe degeneration or even death.

Four to six hours after the skin application of lewisite the liver shows increasing congestion with progressive cloudy swelling. The gall bladder and bile ducts are usually distended with a jelly-like mass streaked with blood at its periphery. Hæmorrhages may be large in the gall bladder wall and are associated with opaque, often wrinkled areas. The common bile duct shows dilatation in its upper part, the terminal portion being dark red in colour. The neighbouring

PATHOLOGICAL CHANGES INDUCED BY LEWISITE

Fig 1 —Gall bladder and bile ducts of goat, 24 hours after skin application of lewisite
Intense congestion and hæmorrhagic necrosis, stopping abruptly at the ampulla
of Vater

Fig 2 —Fatty necrotic liver and hæmorrhagic gall bladder of guinea pig 16 hours
after skin application of lewisite

Fig 3 —Confluent necrosis of liver and coagulation necrosis of gall bladder of guinea
pig, 48 hours after skin application of lewisite

Fig 4 —Necrosis, sloughing and hemorrhage of wall of stomach of rabbit 24 hours
after oral administration of lewisite oxide

duodenum and sometimes the soft structures along the course of the common bile duct are characteristically infiltrated with blood. Microscopically, the liver shows little change beyond marked congestion of all its blood vessels and cloudy swelling of the liver cells. An occasional animal presents small areas of necrosis. The bile ducts within the liver appear normal and there is no evidence of intrahepatic bile stasis. Necrosis and hæmorrhage have destroyed a large portion of the gall bladder mucosa: elsewhere the epithelial lining is denuded. The bare submucous coat is œdematous and infiltrated with polymorphonuclear leucocytes. Lymphatics are distended with cellular lymph. The terminal common bile duct presents a similar appearance, its lumen filled with a necrotic mass of desquamated epithelium, blood and leucocytes. Massive hæmorrhage occupies the wall of the bile duct and can be traced into the outer coats of the duodenum and the lesser omentum. Not infrequently the papilla of Vater is partly destroyed and is very œdematous. The orifice is plugged with soft necrotic material.

From **twelve hours** onwards the liver becomes mottled with tiny yellow or greyish areas several mm. in diameter, fairly uniformly distributed but most constant in the right and left middle lobes and close to the gall bladder. These are often rimmed by a narrow scarlet zone of intense congestion. They assume a dark green colour in formalin. By 24 hours necroses are well developed ; they sometimes coalesce to form infarct-like areas. The smaller lesions are pear-shaped or irregularly rounded, often intensely bile stained and frequently surrounded by a narrow zone of " hyaline " or fatty liver cells. Most of the necrotic areas are in close association with portal canals, in the midst of which are damaged bile ducts and portal veins. The latter are sometimes thrombosed. Damaged bile ducts may be ruptured in one place or lined partly by normal, partly by necrosed epithelium, so that obviously there has been effusion of bile. Closer study of necrotic areas allows of their division into two well defined types, recent and advanced. (1) The recently formed type consists of pale-staining, shrunken cells still arranged in trabeculæ, with normal sinusoids between. Diffuse staining with trypan blue given during life is striking. Nuclei are pyknotic or missing. A narrow peripheral ring of swollen, deeply staining, acidophilic liver cells completely surrounds the inner zone. Nuclei are normal here or pale and swollen. (2) The advanced type shows very swollen, completely vacuolated liver cells, with their trabecular arrangement lost and the sinusoids obliterated. The resemblance to vegetable tissue may be striking (*cf.* fig. 8). Karyolysis is prominent. Around this area is a narrow zone, two or three liver cells thick, of coagulation necrosis. Gradations between (1) and (2) can be made out, but it is usual for type (2) necroses to outnumber the type (1) variety. It would appear, therefore, that there is rapid progress from coagulative to cytolytic necrosis once the lesion gets going. Hæmorrhage is rare in association with necrosis,

probably because of the tendency to thrombosis in the associated vessels. Polymorphonuclear leucocytes migrate from the dilated sinusoids around necrotic areas about the 24th hour but are seldom numerous and do not penetrate far into the dead tissue. Kupffer cells show no evidence of proliferation at this stage but may appear abnormally prominent and stellate from attachment of their processes to the walls of the dilated sinusoids.

The gall bladder and common bile duct lesions reach their maximal extent during this period, assuming a plum-coloured hue which is very striking (figs. 1, 2, 5 and 7). The condition may well be described as acute cholecystitis and choledochitis due to a chemical irritant. No microscopical evidence of bacterial invasion was obtained.

During the **second and third days** the liver necrosis appears to have increased, many of the small areas coalescing to form large infarct-like masses, especially near the margins of the lobes (fig. 3). Hæmorrhage and necrosis are still prominent in the gall bladder and common bile duct (fig. 6). Most of the liver necrosis is now of the acute cytolytic variety, whilst polymorphonuclear cell invasion progresses at the edges of the necrotic regions. By the third day the smaller areas of necrosis show evidence of absorption, whilst mononuclear cells, both lymphocytes and macrophages, are increasing in number. Newly formed liver cells around absorbing necroses and bile duct proliferation in portal canals indicate the onset of regeneration. Gall bladder and bile duct lesions are in process of absorption and shrinkage. Regeneration of the mucosa proceeds by proliferation of the lining epithelial cells of its glands and crypts, which have escaped the initial damage.

From the **fourth day** onwards recovery is rapid in surviving animals. Necrotic areas are usually small and few by the fifth day, although in some cases they may still be large and liquefying. Necrosis and hæmorrhage in the bile passages are seldom seen after this time. Microscopically the picture is typical of liver repair. The small areas of necrosis consist of poorly defined masses of faintly eosinophilic granules, representing damaged liver cells in process of removal. A few leucocytes and macrophages are also present. The normal liver cells around show very large nuclei with occasional mitotic figures. Fibroblasts are few in number around small necrotic areas but more numerous in the confluent lesions. Bile duct proliferation may be a striking feature in such cases, and the newly formed buds growing out from the parent bile ducts present many mitotic figures. The connective tissue in the portal canals is more cellular and lymphatics are dilated. Bile duct changes are especially well marked at the margins of necrotic areas where these impinge on portal canals. Solid buds lie side by side with ducts with narrow lumina, and appear to be advancing towards foci of proliferating liver cells, which are generally numerous near by.

Rapid absorption of necrosed tissue and effused blood, with

PATHOLOGICAL CHANGES INDUCED BY LEWISITE

FIG. 5.—Portion of wall of gall bladder of guinea-pig, showing hæmorrhage and necrosis. 24 hours after skin application of lewisite. H. and E. ×16.

FIG. 6.—Transverse section of common bile duct of guinea-pig, showing intense hæmorrhage and necrosis of the wall generally and early inflammatory œdema of the subserous coat. 48 hours after skin application of lewisite. H. and E. ×50.

FIG. 7.—Transverse section of pancreatic portion of common bile duct of guinea-pig, showing hæmorrhage into and partial destruction of the inner coat, and pancreatic hæmorrhage. 24 hours after skin application of lewisite. H. and E. ×50.
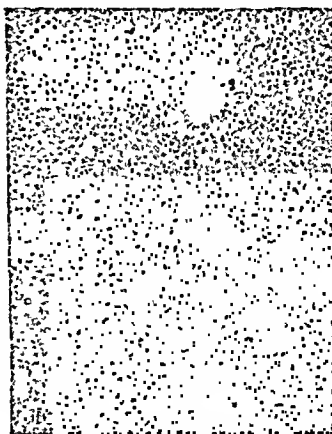
FIG. 8.—Focal cytolytic necrosis of guinea-pig liver, close to portal canal, showing resemblance to vegetable tissue. 48 hours after skin application of lewisite. H. and E. ×80.

proliferation and overgrowth of surviving mucosal epithelium, is responsible for repair in the extra-hepatic bile passages. Necrosis of the common hile duct is seldom seen after the sixth day. Permanent damage to the bile passages of survivors is not common, though occasionally stenosis of the common hile duct or late perforation of the gall bladder may develop. Delayed arsenical poisoning is sometimes seen.

Similar liver and biliary lesions develop in the guinea-pig following subcutaneous and intra-peritoneal administration of small amounts of lewisite. In the rabbit, goat and dog, too, skin application or intravenous injection leads to the same changes, but these are less constant and not nearly so marked as in the guinea-pig. The rat and mouse show no characteristic features in their bile passages and the liver structure is only mildly damaged, presenting cloudy swelling and slight fatty degeneration.

Urinary system. Renal damage is seldom severe. Rabbits given intravenous injections of 0·005 c.c. of lewisite show cloudy swelling of the tubules after one hour, patchy necrosis of tubules with some desquamation of lining epithelium and cast formation after 24 hours. Glomerular congestion and effusion of fluid into the glomerular space are not infrequent in the early stages and small hæmorrhages are sometimes found. The condition is thus one of parenchymatous degeneration or nephrosis. The condition seems to clear up in about four days.

Sometimes a much more severe picture develops which closely resembles that of acute arsine poisoning. The glomeruli then stand out as swollen, congested spheres, their spaces partly filled with altered hæmoglobin. A granular eosinophilic exudate occupies the lumina of many convoluted tubules and Henle's loops and the collecting tubules show large deeply eosinophilic or brown casts or cylinders. The epithelial cells of many of these convoluted tubules are degenerated or necrotic. Pigment granules are present in some, while calcium casts form later in scattered necrotic tubule cells. The reticulo-endothelial cells of these animals are loaded with hæmoglobin-derived pigment and the blood may be hæmolysed. A similar picture was seen in a few mice but not in rabbits or rats. Obviously an acute hæmolytic crisis can be precipitated by lewisite administration.

In the most severe cases, a condition of symmetrical renal cortical necrosis may develop, but this is rare.

Alimentary system. Absorption of lewisite by routes other than the alimentary canal leads to serous and mucosal hæmorrhages and erosions in the stomach and intestines of the cat, dog and goat; the guinea-pig, rat and mouse less often show these features.

In view of the severe damage occurring in the bile passages it is of some interest that lesions are hardly ever seen in the duodenum, which receives the bile. Either there is complete hile stasis, with failure of bile to reach the duodenum, so that the irritant has no

chance of damaging the duodenal mucosa, or the agent is inhibited either in the bile itself or when it reaches the duodenum. The latter possibility seems unlikely if the irritant is lewisite oxide, for this compound exerts severe effects on the duodenal mucosa when directly applied to it. We believe there is complete stasis of bile in the biliary passages, especially as the lumen of the papilla of Vater is invariably blocked by a plug of necrotic, hæmorrhagic material.

Central nervous system. No changes are found apart from congestion of the membranes and sometimes œdema of the brain. Special methods of investigation of the nervous tissues fail to disclose any severe damage in the nerve cells or tracts.

Other organs. The spleen is usually small, collapsed, pale and almost bloodless, with a dry wrinkled capsule. Lymph glands are sometimes hæmorrhagic. No changes are found in the ductless glands apart from inconstant adrenal congestion and hæmorrhage. Reproductive organs are normal.

DISCUSSION

Although the properties of lewisite I have been known since the 1914-1918 war, detailed information about its action is scanty. Vedder (1925) discusses its effects in dogs, recognising a powerful vesicant action on the skin and damage to the respiratory tract. He noted intense congestion of the liver and kidneys in fatal cases. The skin reaction in man has been described in detail by Warthin and Weller (Warthin, 1926). German and Italian text-books on chemical warfare are singularly uninformative on the subject, although all describe the skin and lung lesions and are fully aware of the high toxicity. It is not our intention to describe the complete picture of lewisite poisoning at this stage, since an appreciation of disturbances of circulatory function, discussed in another paper, is essential to the full story.

Our pathological investigations leave little doubt that lewisite and allied compounds exert, when absorbed, a serious action on blood vessels and certain highly specialised cells such as the epithelium of the liver and bile passages. There is evidence, too, of a damaging effect on the red blood corpuscles, which may at times lead to an acute hæmolytic crisis. Renal tissue is not affected to any great extent, but this is most probably due to partial detoxication of the circulating compound in the course of renal excretion. There is every reason to believe that most if not all types of cells are killed, provided the poison reaches them in sufficient amount. Variation in susceptibility of tissues and organs is probably an expression of differences in excretion and detoxication associated with route of administration, rate of absorption, dosage and other unknown factors. The form in which lewisite circulates in the blood or tissue fluids is not known for certain, though it is most likely hydrolysed to " lewisite oxide " at an early stage of absorption. It was shown by the late Surgeon-Lieut. R. M. Calder that this compound is mostly attached to the red corpuscles during the first 24-48 hours after absorption, though some

is carried in the blood plasma and an increasing amount can be detected in the plasma in the later stages. A striking feature is the excretion of a highly toxic derivative, most likely lewisite oxide, by way of the bile, producing severe damage of the liver cells and the biliary passages. Such effects are furthered by spasm of the terminal common bile duct, leading to bile stasis and no doubt retention of the toxic products. We have been impressed by the similarity of the liver lesions with those so easily induced in small animals by simple obstruction of the common bile duct. Spasm, too, is a feature in the sphincteric regions of the alimentary canal after ingestion of lewisite-contaminated food and may be responsible for the localisation of lesions to the stomach wall. Bronchial spasm is likewise a prominent finding when lewisite vapour or droplets are inhaled.

Vascular injury is perhaps the most constant sign of poisoning with the lewisite group of compounds. It is partly responsible for blistering and the rapidly progressive œdema of contaminated skin, changes often accompanied by gross hæmorrhage in the deeper tissues. Effusions into serous sacs, pulmonary œdema after inhalation or from skin absorption, hæmorrhages in the endocardium, pericardium, alimentary canal wall, serous membranes and other situations are all manifestations of vascular damage. The site of action is chiefly the capillaries and the lewisite poisons are essentially capillary poisons. Much of the characteristic picture of intoxication can be explained by this simple generalisation.

Many of these pathological changes are similar to those occurring in arsenical poisoning and no doubt arsenic is responsible for the effects common to the group. If that be so, it would seem that the arsenic has been made more potent than is the case with simple arsenical inorganic compounds such as arsenious oxide, for the fatal doses (and arsenic content) of the lewisite group are much smaller than those for the inorganic compounds. It is possible that the organic forms penetrate cells more easily than the inorganic, being highly lipoid-soluble, and that minute amounts of arsenic are liberated within cells to bring about the injurious effects. An essential feature for toxicity appears to be the presence of a trivalent arsenic atom, for if lewisite I be converted into 2 chlorovinylarsonic acid, as follows,

$$CHCl = CH-As{\Large\langle}^{Cl}_{Cl} \longrightarrow \begin{array}{c} CHCl = CH-As = O \\ {\Large\updownarrow} \\ CHCl = CH-As{\Large\langle}^{OH}_{OH} \end{array} \Bigg\} \longrightarrow CHCl = CH-As = O{\Large\langle}^{OH}_{OH}$$

Lewisite I   "Lewisite oxide"   2 chlorovinylarsonic acid
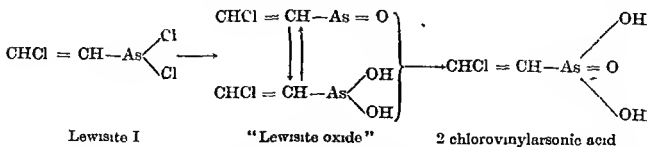
toxicity falls considerably with the production of a pentavalent atom. In this way, lewisite resembles other trivalent organic compounds and no doubt this principle governs the process of detoxication.

SUMMARY

A group of poisons is described, the members of which exert an action typified by that of 2 chlorovinyldichloroarsine (lewisite I). When applied to the skin they produce blistering and necrosis. They severely damage the eyes, are highly irritating to the nose and respiratory passages when inhaled, and destroy the mucous membrane of the mouth, stomach and intestines when ingested. They are easily absorbed into the circulation, thereby exerting serious action on the blood vessels and various organs. Excretion occurs by way of the bile passages and kidneys, in the course of which much damage is produced in the liver, bile ducts and gall bladder but less in the kidneys and urinary tract. Capillary permeability is greatly increased and the massive depletion of circulating blood plasma leads to a condition not unlike burn shock : this we have called " lewisite shock ". It may terminate life in a few hours or days. Death may also result from hepatic insufficiency, either in the initial stages—most often the first week—or after some delay. Occasionally an acute hæmolytic crisis·is precipitated, with a pathological picture identical with and as fatal as that due to arseniuretted hydrogen.

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AN ACCOUNT OF THE PATHOLOGY OF SOME CASES OF *CL WELCHII* INFECTION

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(PLATES LX-LXIV)

THE following paper is an account of the histology of muscle biopsies taken from war service wounds infected with pathogenic Clostridia, with an attempt to explain the extreme rapidity of spread of gas gangrene   In addition a report of the post mortem and histological appearances in three fatal cases of clostridial infection is appended, and certain changes not hitherto noted in these conditions are described

The material at my disposal consisted of muscle biopsies from 120 service casualties   Eleven of these patients were critically ill and, in the opinion of surgeons with extensive experience in the last war, merited the diagnosis of gas gangrene   Fifty two other cases showed varying degrees of toxaemia accompanying muscle damage   Clinically these were labelled " mild " and " moderate " grades of gas gangrene   Of the three cases coming to autopsy, two were service casualties and one died of " natural causes "   In all three *Cl welchii* was practically the only clostridium found   For obvious histo pathological reasons " clostridial myositis " will be used as a synonym for the older term " gas gangrene "

LOCAL CHANGES

Two types of lesion require description   that seen in certain of the " mild " cases and the other found in patients who were critically ill

Immediately adjacent to the wound surface in both types there is a zone of necrosis involving all elements   The muscle fibres most commonly show a coagulative necrosis with loss of striation and nuclei   Although these fibres persist for some time they eventually become thinned and disappear (fig 1)   In other cases there is a similar loss of striation and nuclei but the fibres show a tendency to fuse as if the necrosis was more liquefactive (fig 2)   Vacuolation is a prominent feature in both types of necrosis and is accompanied by invasion of the fibres by Gram positive bacilli in large numbers   The endomysial tissues and sarcolemma disappear at an early date

and this dissolution also affects the vascular and nervous structures, although these tend to retain their structure long after the finer connective tissues have disappeared. Occasionally the proximal part of this necrotic area shows an intense fibrinous reaction, the muscle fibres being bound together by dense Gram-positive strands (fig. 3).

Beyond this area of advanced necrosis the appearances differ in the two types of case. In the " mild " case there is a zone of intense leucocytic reaction and congestion and no evidence of spread of infection in the more healthy muscle. Very different appearances are seen in the severe cases. Here, beyond the zone of advanced necrosis, the muscle shows changes which at first sight appear to be due mainly to œdema. This transition from one zone to the other is quite abrupt. In this earlier lesion the muscle fibres are widely separated from each other. Striations are still visible in many, but nuclei are frequently absent and where present are usually pyknotic. The sarcolemma is fragmented and seems to dissolve slowly away. Interstitial connective tissue fibres are separated and often " exploded " in appearance (fig. 4). In some areas they are completely fragmented and in process of disappearing. The fibrocyte nuclei have mostly disappeared and those which remain show pyknosis and karyolysis. Fine reticulum is absent in the muscle adjacent to the zone of advancing necrosis. Beyond this it shows a loss of its argyrophil property and this is followed by its breaking up into granules and ultimate disappearance. Sometimes these changes are limited to about a dozen adjacent muscle fibres. Gram-positive bacilli are found in large numbers in this region but they are confined to the connective tissue planes and show no sign of invading the muscle fibres.

This zone merges insensibly into an area where the œdema is accompanied by intense congestion (fig. 5). Separation of the muscle fibres is still marked but there are fewer signs of necrosis, although many nuclei, both of muscle and connective tissue fibres, have disappeared. There is variability in the staining power of fine reticulum and some breaking up of collagen fibres. Striations are usually well defined. It is the vascular tissue, however, which shows the main changes. Hæmorrhages are common and there is extreme vasodilatation affecting mainly capillaries and venules. The diameter of these vessels is frequently 5 or 6 times that of associated arterioles and they are stuffed with red cells. Many of these vessels are thrombosed. Some contain ordinary fibrinous thrombi but the capillaries often contain hyaline thrombi formed by conglutination of red cells. This congestion usually extends for a considerable distance—2 or 3 cm.—along the muscle, and clostridial organisms can be found in large numbers in the first part of this area (fig. 6), although I have not been able to demonstrate them in the tissues beyond. The zone of congestion would appear to mark the spreading edge of the lesion (fig. 7), but it is rarely clear-cut, and in a number of cases individual swollen coagulated fibres resembling those described by

CLOSTRIDIAL MYOSITIS

Fig. 1.—Section of muscle from zone of advanced necrosis showing discrete necrotic fibres undergoing gradual solution. The apparent endomysial tissue consists of columns of bacteria. Hæmatoxylin and eosin. × 100.

Fig. 2.—Muscle from area of advanced necrosis showing fusion of fibres. Hæmatoxylin and eosin. × 100.

McNee and Dunn (1917) can be traced for varying distances among otherwise healthy muscle fibres (fig. 8).

Leucocytic emigration is practically absent except in the last zone and even in this area it is extremely scanty. Where the above changes are limited to a small number of adjacent fibres, however, a marked leucocytic reaction can be seen in the relatively healthy muscle running parallel with the necrotic fibres.

It frequently happens that the clostridial infection is not strictly confined to muscle but spreads to surrounding tissues. Adipose tissue is often attacked and most commonly shows complete necrosis of the cell envelopes and karyolysis of the nuclei. Occasionally the cells show the typical cloudy appearance of fat necrosis. Large numbers of Gram-positive bacilli are usually found among these fat cells.

Lymphatics take part in the local vaso-dilatation and often reach immense diameters. Gram-positive bacilli can frequently be seen within them. The local lymph glands show diffuse hæmorrhagic destruction of the medullary tissues.

Hæmolysis is not a prominent feature in the biopsies but the red cells show a tendency to clump and fuse in the dilated capillaries. There is little evidence of the products of blood destruction, but small spherical masses of bright golden pigment, approximately 15-30 μ in diameter and having a rosette-like structure, are frequently seen in necrotic muscle and fat. They fail to give an iron reaction.

Commentary

One of the chief problems in regard to the local lesion is the extreme rapidity of spread of muscle necrosis and it is obvious that any explanation of this phenomenon must be based upon an interpretation of the microscopical changes observed in biopsies.

The initial necrosis is so advanced and the appearances differ so much from succeeding areas that one can only conceive that the results are not entirely due to the infection. It seems reasonable to suggest that this necrosis is mainly due to injury and that the disappearance of collagen and reticulum is the result of subsequent clostridial proliferation. In the mild cases this bacterial growth appears to remain as a contamination.

If this is true then we must look to succeeding zones for the true picture of clostridial myositis. One of the main features in these areas is the separation of muscle fibres from the interstitial connective tissue. McNee and Dunn suggested that this is due to a toxic fluid, formed from the already necrotic tissue, diffusing along individual fibres, separating them from their vascular supply and thus by a combination of direct and indirect action causing their death. Necrosis extending along individual fibres was taken as an indication of proof of their theory.

This view cannot be accepted without some hesitation. Separation

of muscle fibres from their connective tissue sheaths without necrosis can be seen in other infective conditions and it is the rule in regenerating muscle fibres. It has been demonstrated by many authors that, until a very late stage, the clostridia are confined to the connective tissue planes. To be consistent with the theory of Dunn and McNee and to account for the death of isolated fibres the toxin would have to spread along individual fibres without lateral diffusion. Otherwise we should expect adjacent fibres to show degenerative changes. While there can be no doubt that the clostridial toxins act directly on the muscle fibres, this does not altogether explain the rapidity and peculiar nature of the spread of necrosis. The vascular changes in the tissues furthest from the wound surface cannot be without effect. Emrys-Roberts and Cowell (1916-17) declared that vascular damage only occurs at a late date, but Bashford (1916-17, 1919), who made an extensive examination of gas gangrene material, was equally convinced that it is a constant feature and one which is responsible for much of the necrosis. In the cases under consideration vascular changes are marked, thrombosis is common and it is difficult to believe that the intense capillary engorgement could result in anything but stasis. The fact that the changes are frequently confined to a few adjacent fibres and that they extend along individual fibres points to some anatomical cause. It is significant that Le Gros Clark (1945) has shown that the vascular anastomosis in muscle is functionally more apparent than real. He has demonstrated that it requires several days for a collateral circulation to be established in a muscle whose nutrient supply has been blocked, and even then the circulation is scarcely adequate. We know that the vascular supply runs longitudinally, parallel with the fibres, and it seems reasonable to suggest that we have here an adequate explanation of the spread of necrosis without recourse to a hypothetical geometrical diffusion of toxin. It is suggested that the changes noted in the muscle fibres are largely the result of thrombosis and stasis in the related vessels.

Systemic Changes

The material for the following description is drawn from three post-mortem examinations. In two, death was due to clostridial infection of a gun-shot wound : the other was a case of terminal clostridial infection of the bladder following compression myelitis.

Case 1

The subject was a young soldier with a gun-shot wound on the outer side of the right arm and the typical appearance of gas gangrene was apparent on opening up the wound track. Bacteriological and histological examinations confirmed the clinical diagnosis. The post-mortem was performed by Professor G. Haswell Wilson 3 hours after death. The heart contained abundant agonal clot but was otherwise normal. Both pleural cavities contained 2-3 pints of clear straw-coloured fluid. The lungs were deeply congested and the lower

lobes were waterlogged. There were small petechiæ on the pleural surfaces and larger areas of hæmorrhage resembling infarcts in the sub-pleural tissues. The bronchi and trachea contained abundant frothy fluid.

A little free fluid was found in the peritoneal cavity but no abnormality could be found in the stomach or intestines. There was some congestion of the centres of liver lobules. No significant change could be seen in the spleen. The kidneys were enlarged and pale but the glomeruli stood out as minute scarlet points in the cortex.

The brain and its membranes were intensely congested. No abnormality could be found in the grey matter but the white matter and the cord were much softer than normal.

Case 2

This subject, a soldier aged 28, had sustained a gun-shot wound in the left upper arm. He was treated with sulphonamides and penicillin but developed signs of gas gangrene in hospital and died four days later. Fifteen days elapsed between the date of injury and death.

A post-mortem examination was performed six hours after death. The subject, a well-built adult male, showed a penetrating wound extending from the left upper arm through the deltoid muscle, fracturing the head of humerus and upper part of scapula and emerging through the supraspinatus muscle. Gangrene could be seen extending into the muscle surrounding the wound track. Bacteriological examination confirmed the diagnosis. No obvious abnormality could be found in the cardiovascular system. Both pleural cavities contained about three pints of clear fluid. The lungs were oedematous and patchily congested. No significant abnormality could be seen in the abdominal organs. The brain was irregularly congested but there was no sign of softening.

Histology

The appearances were very similar in the two service cases and the following account of the histology is taken mainly from case 1. Paraffin sections were stained with hæmalum and eosin, frozen sections with eudan IV. The central nervous system was examined by the methods of Marchi, Weigert-Pal and Alzheimer.

Lungs. Pieces of tissue were examined from both hæmorrhagic and non-hæmorrhagic ereas. In the latter, paraffin sections showed intense congestion and alveolar œdema. Large numbers of " heart failure " cells were present in the alveoli ; they contained both iron-reacting granules and tiny globules staining red with sudan dyes. The hæmorrhagic areas had the typical appearance of infarcts. Thrombosis wes common around these areas. In both infarcted and non-infarcted areas frozen sections showed that many of the vessels contained fat emboli.

Kidneys. The cortical tissue was much congested. Glomeruli were enlarged and the tufts in most instances filled their capsules. Glomeruler hæmorrhages were common. In meny cases the cepsular epithelium was cuboidal. There was some dilatation of the limbs of Henle and the epithelium showed very slight fatty degeneration. Fat emboli were present in a few glomerular tufts and adjacent vessels.

Nervous system. Chenges in this region were marked in the case without fracture and the patient beceme blind several hours before death. Exemination of sections suitebly steined showed that many parts of the brain and cord were demyelinated (fig. 12). This was most marked in the optic nerves. Other areas showed myelin degeneration. The vessels of the cord and brain were much congested and many were thrombosed, the thrombi containing many polymorphs. Fat emboli were common (fig. 10). Some of these seemed to

consist of clusters of tiny fat particles (fig. 9). In several capillaries the endothelial cells had phagocyted considerable numbers of these fat particles (fig. 11).

Spleen. The sinusoids were greatly dilated. The red cells showed a tendency to clump and fuse and many of the fused masses had been taken up by macrophages. There was, however, little evidence of gross hæmolytic activity.

Liver. The central veins and their tributaries were dilated and around some of them the liver cells had become necrotic. The rest of the liver tissue showed occasional areas of very slight fatty deposit but this had no distinctive distribution.

Adrenals. Degenerative changes were noted in the cortical cords of cells resulting in an apparent formation of glandular lumina.

In none of these organs was there any evidence of embolic or septicæmic spread of clostridia.

Case 3

J. W., aged 29, a soldier, was admitted to the Queen Elizabeth Hospital, Birmingham, on 23.10.44. A year previously a diagnosis of sarcoma of the left ilium had been made. Deep X-ray therapy had relieved the symptoms but these returned and on 24.10.44 paraplegia with retention of urine developed. The temperature began to swing between 97° and 104° F. for no apparent reason. On 3.11.44 jaundice appeared, by 14.11.44 the patient complained of diplopia and on 15.11.44 he died.

Post-mortem examination

Externally there was marked wasting, jaundice and abdominal distension. Large quantities of free gas escaped when the peritoneum was opened.

No abnormality could be found in the heart or pericardium. Both pleural cavities contained about 3 pints of slightly blood-stained fluid. The lungs were deeply congested and œdematous and many small infarcts were present. Masses of semi-necrotic white growth filled the upper anterior mediastinum. The bodies of the 2nd, 3rd and 4th dorsal vertebræ were invaded and the cord was compressed. The apex of the left lung was involved in growth but this was obviously metastatic.

The stomach and intestines were normal, although distended. Almost the whole of the liver was honeycombed with gas bubbles. The spleen was of septic type. The kidneys, which were enlarged, showed intense pyelonephritis, while the cortex was riddled with small holes. The bladder wall was thickened, hæmorrhagic and extensively ulcerated.

The blade of the left ilium was diffusely softened by new-growth which bulged into the peritoneal cavity. The tumour measured approximately 4 × 3 in. but its outlines were very diffuse. The pre-aortic lymph glands were infiltrated.

No gross abnormality could be found in the brain apart from small irregular areas of congestion in the white matter.

Histology

Iliac tumour. Sections showed that this was a malignant endothelioma of Ewing type.

Lungs. The tumour here was obviously a metastasis from the iliac growth. The remainder of the lungs showed marked œdema and congestion, with many "heart-failure" cells. Frozen sections showed that fat emboli were even more numerous than in the previous case. Small colonies of Clostridia were present in the pulmonary vessels but were not associated with the fat emboli, infarcts or tumour. No reaction could be seen around these bacteria and the appearances suggested that their presence was a post-mortem or agonal phenomenon.

PLATE LXI

CLOSTRIDIAL MYOSITIS
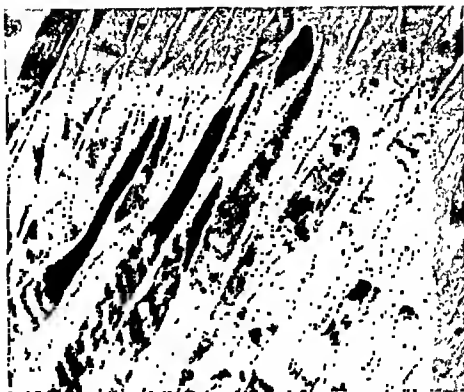
FIG. 3.—Necrotic muscle fibres embedded in fibrin network. Gram's stain. ×100.

FIG. 4.—A bundle of muscle fibres showing less advanced necrosis The fibres are widely separated and the connective tissue has been broken up A marked leucocytic reaction is present along the (upper) lateral margin of the bundle. Hæmatoxylin and eosin. ×75.

CLOSTRIDIAL MYOSITIS
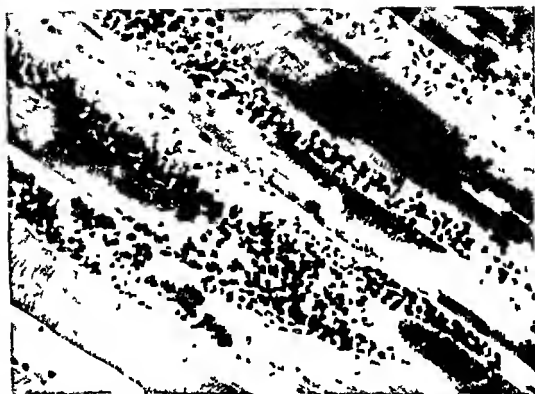
Fig 5 —Muscle from zone of congestion Hæmorrhage is marked and although striations are preserved the connective tissue has disappeared and most of the muscle nuclei have gone Hæmatoxylin and eosin   ×200
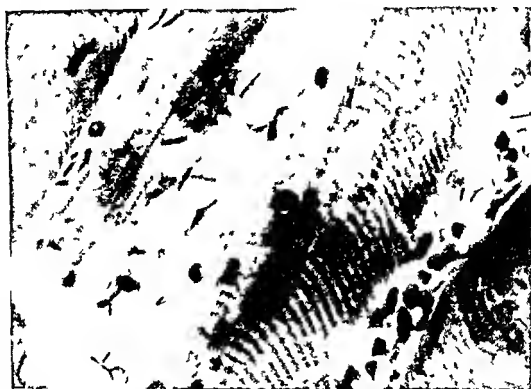
Fig 6 —A section of the same part of the muscle shown in f g 5 stained by Gram s method   Gram positive organisms of clostridial type are numerous   ×400

CLOSTRIDIAL MYOSITIS

Fig 7 —Muscle beyond the limits of clostridial invasion The congestion and hæmorrhage are still marked Hæmatoxylin and eosin   × 50
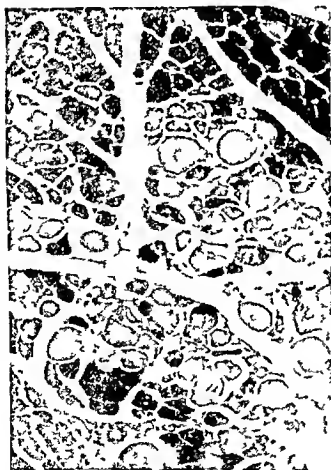
Fig 8 —Section of muscle showing an appearance occasionally seen among fibres distant from the wound surface Note the wide separation of muscle bundles and the isolated swollen necrotic fibres Hæmatoxylin and eosin   × 100

Fig 9 — A large fat embolus consisting of many adhering small globules in a medullary vessel of the spinal cord Case 1 Hæmatoxylin and Sudan IV   × 400

CLOSTRIDIAL MYOSITIS
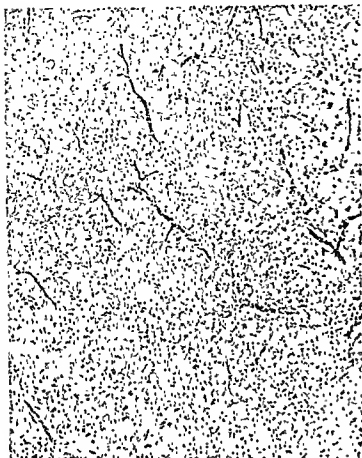
FIG. 10.—Section of brain from case 2. All the capillaries are filled with columns of fat. Hæmatoxylin and Sudan IV. ×75.

FIG. 11.—Section of brain from case 1 showing a capillary in which the endothelial cells are laden with fat particles. Hæmatoxylin and Sudan IV. ×400.
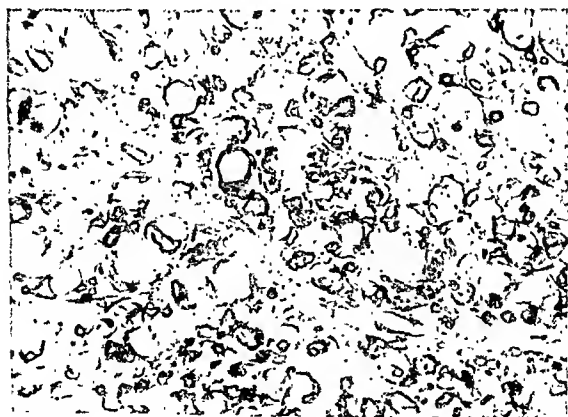
FIG. 12.—High power view of the periphery of the thoracic spinal cord of case 1, stained Weigert-Pal. Note irregularity of myelin sheaths and partial or complete disappearance of many others. ×420.

Kidneys. The spaces noted *post mortem* were obviously due to gas bubbles and masses of clostridial organisms lay in close relation to them. No sign of reaction could be seen around them. There was marked subacute pyelitis and clostridial organisms were present in large numbers in this situation. The tubules showed widespread necrosis and some of them contained pigmented casts. Cuboidal transformation of the capsular epithelium was present in some of the glomeruli and a few of them contained small fat emboli.

Liver. The liver cells were very degenerate but there was no sign of necrosis. Masses of clostridia surrounded the gas bubbles. As in the lungs this appeared to be a post-mortem or agonal invasion.

Nervous system. Demyelination and myelin degeneration were found in many parts of the brain. Fat emboli were extremely common and were almost always found near the areas of myelin change. Thrombosis was also common.

No significant change could be found in the heart, spleen or adrenals.

Commentary

Prior to short communications by Frazer *et al.* (1945) and Cooke *et al.* (1945) no mention has been made of fat embolism in relation to clostridial infections. Kettle (1919), in one of the few detailed pathological studies of gas gangrene, makes no mention of lung lesions. Eliot (1927) described the occurrence of subpleural hæmorrhages in human cases but did not make a detailed investigation of the cause. Gordon *et al.* (1940) described symptoms and post-mortem changes in animals injected with type A *welchii* toxin which suggest fat embolism. The symptoms were acute dyspnœa, blood-stained froth at the nostrils, muscular incoordination and weakness. *Post mortem* the lungs showed hæmorrhagic œdema. It is interesting to note that in 1902 Westenhoeffer described fat embolism due to the action of gas-forming organisms, but he was of the opinion that it was a post-mortem phenomenon. He does not appear to have identified the organism responsible. Bürger (1910) declared that this explanation was untenable and that fat embolism was an ante-mortem occurrence.

The occurrence of fat embolism in the present cases does not appear to be accidental. It was found in the service casualty where no fracture was sustained and in the case of paraplegia. The question appeared to be finally settled by animal experiment (Cooke *et al.*, 1945). Injections of *welchii* toxin into the thigh muscles of guinea-pigs and rabbits invariably gave rise to pulmonary fat embolism.

At least three possible explanations require consideration. *Welchii* toxin contains a high proportion of lecithinase and this ferment, by its solvent action on the local fat cells, may set the fat free to find its way into the general circulation. The ferment itself may find its way into the blood stream and it has been shown that it will split the blood lipoprotein (Nagler, 1939) and flocculate the chylomicrons (Elkes and Frazer, 1943-44). Either of these reactions may result in the formation of fat emboli, but it requires several hours' incubation for their in-vitro demonstration and fat emboli have been found in guinea-pig lungs one hour after the intramuscular injection of toxin.

Whatever the cause of the liberation of free fat in the blood stream, its marked phagocytosis by the capillary endothelium indicates that the body can deal with a considerable amount of fat in globular form, and although fat embolism is probably of frequent occurrence in a variety of circumstances (Robb-Smith, 1941) it is likely that a fatal issue is rare unless vascular obstruction is extensive.

SUMMARY

1. The histology of the local muscle lesion in gas gangrene is described.

2. Evidence has been found to indicate that capillary and venous thrombosis is one of the main factors responsible for the mode of spread of gas gangrene in muscle.

3. The occurrence of widespread fat embolism in clostridial infections is described in detail for the first time.

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THE PATHOLOGY OF SIMPLE GASTRITIS

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(Plates LXV-LXIX)

THE histological investigations recorded in this paper were undertaken in order to examine the repeated claims made between 1926 and 1935 by Faber (1935) and Konjetzny (1928, 1930, 1934, 1935; Konjetzny and Puhl, 1926) that the structural changes in the gastric mucosa which they describe as gastritis stand in close causal relationship with simple peptic ulceration and gastric cancer.

MATERIAL AND TECHNIQUE

The material investigated comprises two groups of specimens.

1. Twenty presumably " normal " stomachs removed *post mortem* following the injection of formelin into the stomach 15 minutes after death. The degree of autolysis after delay in fixation beyond 45 minutes was found materially to increase the difficulty of histological analysis. After a delay of two hours exact interpretation of histological appearances becomes impossible.

2. One hundred partial gastrectomy specimens.

The " Swiss-roll " technique and the histological methods used in this investigation have already been fully described (Magnus, 1937).

Classification

The investigation was confined to the structural changes found in true inflammatory gastritis, excluding acute phlegmonous gastritis in which bacteria are readily demonstrable. Non-inflammatory gastritis as found in Addisonien pernicious anæmia, confined to the body area of the stomach and involving the whole thickness of its wall but not affecting the pyloro-duodenal region, was described in a previous paper (Magnus and Ungley, 1938) and is not dealt with here. This leaves for consideration the pathology of acute and chronic gastritis, which will be dealt with according to the following classification.

AOUTE EROSIVE GASTRITIS

PYLORIC   DIFFUSE

CHRONIO ATROPHIO   CHRONIO DIFFUSE
PYLORIC GASTRITIS.   ATROPHIO OASTRITIS.
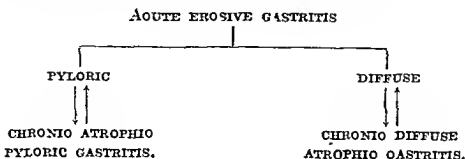
Acute erosive gastritis

In the literature this type of gastritis is usually divided into two types on ætiological grounds. Acute diffuse erosive gastritis is endogenous in origin and intimately related to acute bacterial infection and toxæmia. Nyfeldt and Vimtrup (1932) have described it in children dying from diphtheria and it has been produced experimentally in animals with diphtheria toxin by Enriquez and Hallion (1893), Hayem (1905) and Thomsen (1924-25). On the other hand the available evidence suggests that acute erosive gastritis localised to the pyloric antrum is exogenous in origin and is often associated in its acute and chronic phases with chronic ulceration of the stomach.

Acute erosive pyloric gastritis

The investigations recorded in this paper confirm the close association between this condition and simple peptic ulceration. The transition from acute erosive pyloric gastritis to a similarly localised chronic atrophic gastritis will be described. Emphasis will be laid on the remarkable liability of the acute process when once established in the pyloric antrum to repeated recurrence. The acute and chronic phases are therefore often found together. It is significant that erosive pyloric gastritis was not found in the post-mortem material examined.

Macroscopically, the most striking characteristic of this condition is its sharp localisation to the pyloric mucosa and the first few cm. of the body mucosa, the rest of which appears to be normal. The mucosa of the pyloric antrum is red, œdematous and covered by a patchy layer of adherent mucus. The erosions, ranging in number from a few to over a hundred and in size from a pin's head to several mm. in diameter, are elongated and ovoid and appear to be funnel-shaped because of mucosal œdema. These naked-eye appearances immediately recall Beaumont's description of the stomach of the famous Alexis St Martin after dietetic and especially alcoholic excess (1838), and more recently those described by Wolf and Wolff (1943) in their book *Human gastric function*.

Microscopically there is intense polymorphonuclear infiltration of the interstitial tissue of the pyloric mucosa and the first cm. or so of the body mucosa. Lymphocytes, plasma cells and eosinophils are present in small numbers (fig. 1). This cellular exudate is often perivascular and is most intense in the sub-epithelial layer and in the gastric tips which, in many cases, are distended by serofibrinous exudate (fig. 2). There is widespread capillary hyperæmia of the mucosa. Thrombosis does not occur. The glandular epithelium shows degenerative changes and a variable but often considerable degree of desquamation into the dilated lumen (fig. 3).

PATHOLOGY OF SIMPLE GASTRITIS

Fig. 1.—Acute exacerbation of chronic gastritis. There is intense cellular infiltration of the stroma. Note the sparsity of glandular parenchyma. ×60.

Fig. 2.—Exudate in gastric tips. ×125.
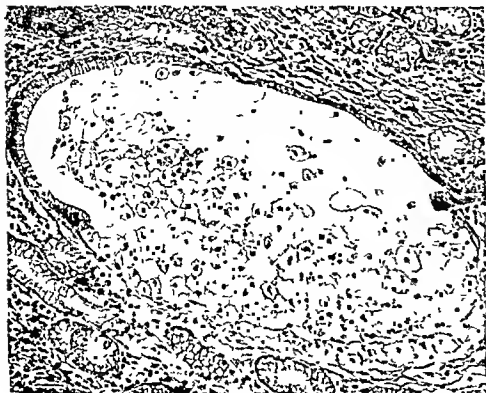
Fig 3.—Cystic gastric gland showing desquamation and degeneration of epithelial cells around part of the circumference. ×140.

All sections stained with hæmatoxylin and eosin.

The surface epithelium shows striking changes. The cells are flattened and cuboidal, with darkly staining cytoplasm and frequent mitotic figures. They are often reduplicated to form a layer several cells thick, their limiting membranes becoming blurred and indistinct (figs. 4 and 5). Perhaps the most remarkable change is the presence between the cells of large numbers of vacuoles which contain polymorphonuclear leucocytes (fig. 6). These cells are also present in large numbers in the overlying mucus. These changes in the surface epithelium are only found in erosive gastritis and if accompanied by " erosions " indicate that an acute exacerbation of the inflammatory process has occurred.

The typical erosion involves only the superficial part of the mucosa (fig. 7). If it extends through the mucosa and muscularis mucosae into the submucosa it should be regarded as an acute ulcer. Its floor is composed of fibrinoid material covered by a small amount of exudate containing polymorphonuclear leucocytes (fig. 8). Deep to the floor the interstitial tissue is œdematous and infiltrated by polymorphonuclear leucocytes and lymphocytes. No evidence of fibrosis is seen around the erosion, which presumably heals without leaving a scar. All the erosions seen in the material examined had a floor composed of fibrinoid material and none was so small that it could not have been seen with a hand lens.

In every case the body mucosa, apart from the first cm. or so near the pyloric mucosa, showed no evidence of acute erosive gastritis. In a few cases it showed the changes of diffuse atrophic gastritis, which had presumably been produced in the first place by an acute diffuse gastritis probably endogenous in origin.

Chronic atrophic gastritis

In the majority of cases this condition was localised to the pyloric antrum and was only occasionally found as a diffuse lesion. Histological evidence strongly suggests that it is inflammatory in origin and the result of repeated attacks of acute erosive gastritis. From the material examined it was clearly associated with both simple and malignant ulceration of the stomach and duodenum.

The gastric mucosa in chronic atrophic gastritis may be compared with the heart in rheumatic fever. Each wave of acute inflammation does a little more damage, produces a little more atrophy and reparative fibrosis and, in any particular stomach, the lesion present may be a mixture of the acute and chronic phases of the disease.

Macroscopically, the mucosa in chronic atrophic gastritis, apart from evidence of an acute exacerbation, commonly shows no naked-eye abnormality even though, histologically, a severe degree of gastritis is present. In collaboration with H. W. Rodgers I have been able to obtain partial gastrectomy specimens from patients previously gastroscoped by him, and, in many cases, the negative gastroscopic

appearances gave no hint of the presence of the underlying histological changes (Magnus and Rodgers, 1938).

In one normal stomach and in the normal body mucosa of several partial gastrectomy specimens in which gastritis was localised to the pyloric antrum, a condition has been present which has been described by French writers under the name of état mammelonné. In this condition the gastric areas are unduly prominent, due to an increase in depth of the furrows surrounding them. Konjetzny and others describe this as hypertrophic gastritis (fig. 9). The condition has been found in apparently normal stomachs and measurements of the depth of the mucosa in sections of these unduly prominent areæ gastricæ show that it is no greater than the average depth of normal mucosa. It seems reasonable, therefore, to regard this condition as an exaggeration of the normal mucosal pattern and comparable to lingua plicata.

In some cases of chronic atrophic pyloric gastritis the mucosa of the pyloric antrum is remarkably flat and has a glistening appearance and the areæ gastricæ are almost invisible ; in others the muscle coat in the pyloric antrum seems to be thicker than normal and gives a firm, somewhat rigid feeling to this region. The serosal coat as a rule is normal but may sometimes be congested, especially if there be an acute exacerbation of the inflammatory process in the mucosa. Microscopically there is a striking diminution in the number of mucosal glands present and a corresponding increase in the amount of interstitial tissue (figs. 10 and 11). This glandular atrophy, however, never reaches the extreme degree seen in non-inflammatory atrophy of the body mucosa. Of the surviving glands some may show little change, while in others the lining cells are in various stages of degeneration. Frequently adenoma-like structures are produced by groups of surviving glands being cut off and surrounded by proliferating connective tissue. Occasionally some of the glands dilate to form cysts which are lined by flattened cubical cells—so-called chronic cystic gastritis (fig. 12).

If the body mucosa is involved the changes in the glands are even more striking (fig. 11). In many glands the chief cells are very degenerate or have disappeared ; the parietal cells frequently survive. Sometimes whole glands have disappeared, leaving small isolated groups of parietal cells in the interstitial tissue. In other cases the neck chief cells proliferate and grow down into the glands, replacing the destroyed chief cells, so that glands are produced which closely resemble pyloric glands (fig. 13).

The interstitial tissue is considerably increased, so that there is little decrease in the thickness of·the mucosa. No such fibrosis is seen in non-inflammatory atrophy of the mucosa. In the normal mucous membrane the interstitial tissue is sparse and consists for the most part of argyrophil reticulum. In chronic atrophic gastritis there is free production of collagen. There is considerable diffuse

infiltration with plasma cells (which predominate), lymphocytes, eosinophils and Russell's body cells. The plasma cells are usually in greatest numbers in the more superficial half of the mucosa, whilst the lymphocytes are most abundant in the deeper half, near the muscularis mucosæ. The lymphocytes may be arranged in lymph follicles, with germinal centres, resting on the muscularis mucosæ. Sometimes the lymph follicles are very numerous and extend throughout the mucosa in all directions. They then frequently make their way through the muscularis mucosæ into the submucosa (chronic follicular gastritis—fig. 14). The cells of the surface epithelium and pits may be normal or may show that state of activity so typical of an acute exacerbation already described. The microscopic bud-like and polypoidal out-growths of the surface epithelium and downgrowths of the pits described by German writers as being such a characteristic feature of this type of gastritis have not been seen in the material examined. Epithelium of the intestinal type is frequently found in mucosa showing the changes of atrophic gastritis; this subject has been dealt with in a previous paper (Magnus, 1937).

ANALYSIS OF RESULTS

The following lesions were found in the hundred partial gastrectomy specimens examined.

Primary or secondary malignant ulceration in	33
Simple gastric ulcer in	46
Simple duodenal ulcer in	10
Chronic atrophic pyloric gastritis without ulceration in	5
Chronic atrophic pyloric gastritis with scars of healed ulceration in	3
Chronic atrophic diffuse gastritis with scars of healed ulceration in	1
Acute erosive pyloric gastritis without ulceration or scars in	2

The forty-six specimens in which there was simple gastric ulceration showed the following additional lesions.

Acute erosive pyloric gastritis with macroscopic erosions and severe atrophic gastritis in	2 (4·3 per cent.)
Acute exacerbation of chronic atrophic pyloric gastritis in	5 (10·9 ,,)
Chronic atrophic pyloric gastritis in	25 (54·4 ,,)
Chronic diffuse atrophic gastritis in	14 (30·4 ,,)

Gastritis was thus present in every case. It was severe in degree, with considerable fibrosis and cellular infiltration. Intestinal epithelium was found in thirty-four cases (73·9 per cent.). In two cases it had replaced almost the whole of the gastric mucosa examined. Puhl (1926, 1927) found macroscopic erosions in the pyloric antrum in 27·1 per cent. of his cases and Konjetzny found this lesion in 100

per cent. The low incidence (4·3 per cent.) in the present series is striking.

The ten specimens in which a duodenal ulcer was found to be present at operation showed :

Mild quiescent chronic atrophic pyloric gastritis, with
    intestinal epithelium, in . . . . 2 (20 per cent.)
Mild quiescent chronic atrophic pyloric gastritis,
    without intestinal epithelium, in . . . 8 (80 ,,)

The mild degree of gastritis found and the absence of erosive gastritis in these cases is noteworthy. Walters and Sebening (1932) also found erosive gastritis to be uncommon in association with duodenal ulcer and chronic pyloric gastritis to be similarly mild in degree. Puhl (1926) and other German writers found erosive gastritis to be associated with chronic duodenal as frequently as with chronic gastric ulceration.

Regarding the specimens in which chronic atrophic pyloric gastritis (5 cases) or acute erosive pyloric gastritis (2 cases) was present in association with chronic gastric ulceration, it would appear that these conditions may give rise to symptoms sufficiently severe to justify partial gastrectomy. In four other cases chronic atrophic gastritis—diffuse in one case, pyloric in the remainder—was present in association with one or more scars of healed ulcers.

Acute erosive pyloric gastritis without chronic ulceration is probably the lesion responsible for what has been called the pyloric syndrome, in which the symptomatology is claimed, to be identical with that of chronic peptic ulceration. Many pathological descriptions of this condition are to be found in the literature. The incidence in partial gastrectomy specimens in this series of acute and chronic gastritis with no evidence of active or healed chronic gastric ulcer is 7 per cent. This figure compares favourably with the incidence of 8·8 per cent. in a similar series of specimens reported by Aschner and Grossman (1933).

Gastritis and gastric cancer

Konjetzny and Hurst have for long presented arguments to show that cancer of the stomach develops on the basis of a chronic gastritis. In his Schorstein lecture in 1929 Hurst stated that a diffuse atrophic gastritis, accompanied by achlorhydria, preceded the development of cancer of the stomach in 75 per cent. of cases and that it should, in fact, be regarded as a pre-cancerous state. Konjetzny (1928, 1934, 1935) strongly supports this view and in addition stresses the severity of the atrophy present in association with carcinoma. He describes numerous changes which he regards as pre-cancerous and in which he has observed transition stages to malignant growth. These hyperplastic changes, so-called atrophic-hyperplastic gastritis,

PATHOLOGY OF SIMPLE GASTRITIS
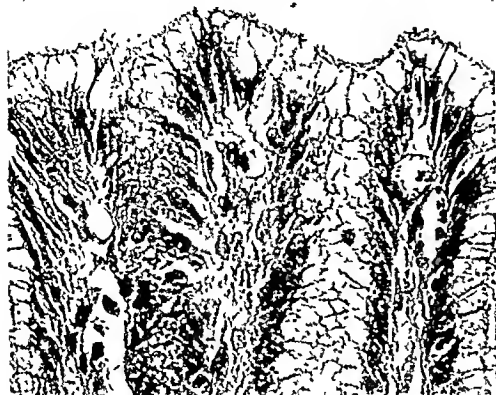
Fig 4 —Normal surface epithelium ×450

Fig 5 —The surface epithelium in acute gastritis ×450
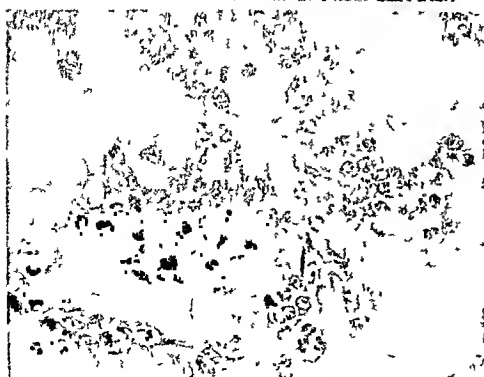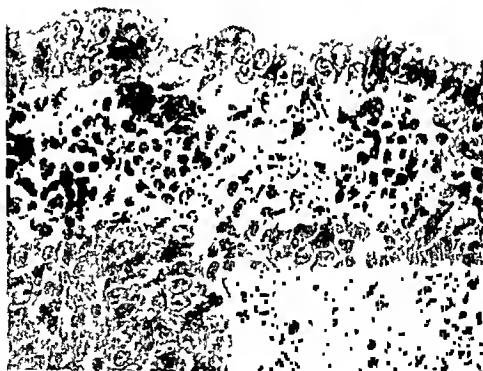
Fig 6 —Vacuoles, containing polymorphonuclear leucocytes, between the cells of the surface epithelium ×500

PATHOLOGY OF SIMPLE GASTRITIS

FIG. 7.—A typical erosion.
×110.

FIG. 8.—The floor of a
larger erosion. ×110.
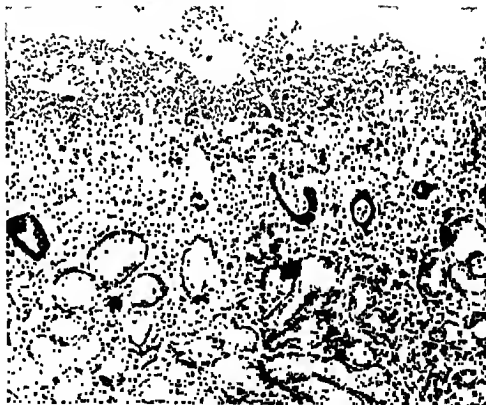
FIG. 9.—État mamme-
lonné. Note the complete
absence of gastritis. ×40.

Fig. 10.—Severe atrophic gastritis involving pyloric mucosa. ×100.

Fig. 11.—Severe atrophic gastritis involving body mucosa. Note the islet of intestinal epithelium (left). ×100.
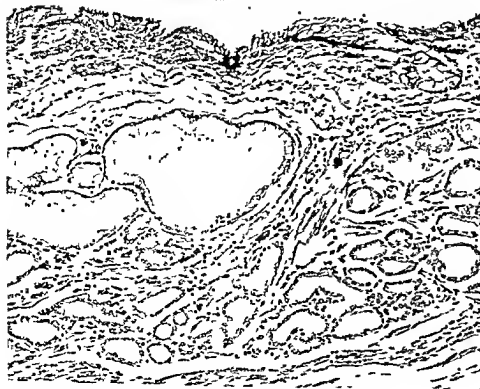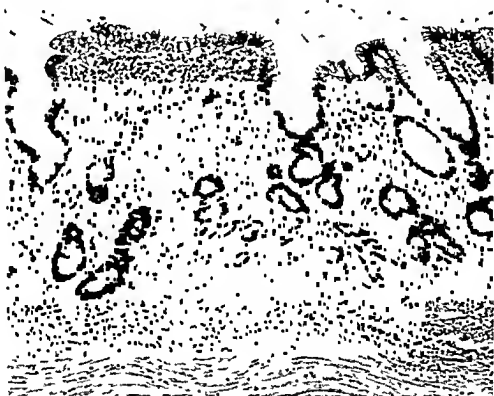
Fig. 12.—Chronic cystic gastritis. ×100.

PLATE LXIX

PATHOLOGY OF SIMPLE GASTRITIS
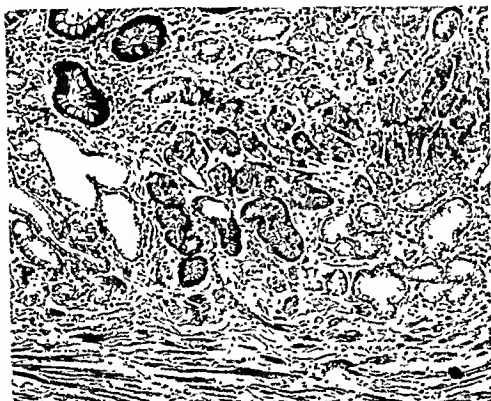
FIG. 13.—An area of pseudopyloric glands around a central area of normal body glands.
× 100.

FIG. 14.—Chronic follicular gastritis.   × 55.

consist of wart-like polypoidal outgrowths of the surface epithelium accompanied, in some cases, by tubular downgrowths of the epithelium into the mucosa and underlying submucosa.

Thirty-three gastrectomy specimens in this series in which adenocarcinoma was present showed the following lesions :

Primary malignant ulceration in 25
Carcinoma arising in a chronic gastric ulcer in . . . 8

Of those, showing primary malignant ulceration there was chronic diffuse atrophic gastritis in three (12 per cent.) and chronic pyloric atrophic gastritis in twenty-two (88 per cent.). In the eight specimens of carcinoma originating in simple chronic ulceration, chronic atrophic gastritis localised to the pyloric antrum was present in all cases. The criteria used for the diagnosis of malignant transformation of a simple chronic ulcer were those published by Hurst and Stewart (1929) and Newcomb (1932-33).

As in the case of simple ulcer the gastritis, when pyloric in distribution, involved the entire pyloric antrum and was not localised to the mucosa in the immediate neighbourhood of the growth. An acute exacerbation of gastritis with the presence of erosions was not observed in any of the specimens. Erosions were present in one case in both body and pyloric antrum, but they were not produced by gastritis. In the case in question the submucosal lymphatics were filled with growth throughout the portion of stomach involved and in many places growth had extended up from the submucosa into the mucosa and formed small intramucosal nodules of growth over which the mucosa had given way to form erosions. These, macroscopically, resembled the inflammatory erosions of gastritis. In no case were any of the hyperplastic or precancerous changes described by Konjetzny observed.

In the twenty-two cases of primary malignant ulcer in which the gastritis was localised to the pyloric antrum the body mucosa included in the specimens was normal. If, therefore, achlorhydria precedes the formation of gastric cancer as frequently as is stated, it is not due to destruction of the parietal cells in the body mucosa. It may be noted here that in three cases of primary malignant ulcer a fractional test-meal showed free hydrochloric acid before the injection of histamine.

It is worthy of emphasis that in all specimens showing gastric carcinoma there was a singular absence of the widespread non-inflammatory atrophic change in the gastric mucosa which accompanies the achlorhydria of Addisonian anæmia and in no case was there a significant diminution in the total number of parietal cells.

CONCLUSIONS

1. Simple gastritis may be divided into inflammatory and non-inflammatory types which differ fundamentally from each other.

2. Inflammatory gastritis is a lesion characterised in its acute stage by the presence of erosions, exudate in the gastric tips, activity of the surface epithelium, destruction of glands and polymorphonuclear infiltration of the mucosa accompanied by transmigration of the surface epithelium by polymorphonuclear leucocytes. In its chronic state it is characterised by atrophy of the glandular parenchyma, fibrosis of the mucosa and submucosa, intense infiltration of the interstitial tissue by plasma cells and lymphocytes, and metaplasia of gastric to intestinal epithelium.

3. Non-inflammatory gastritis or " idiopathic " atrophy of the stomach is characterised by extreme atrophy of all coats of the stomach wall and is not accompanied by any evidence of past or present inflammation.

4. The chronic stage of inflammatory gastritis is an extremely common condition but in the majority of stomachs it is a quiescent and harmless lesion.

5. At least 10 per cent. of all adults develop a chronic peptic ulcer at some time in their lives and this investigation strongly suggests that such lesions arise on the basis of an erosive gastro-duodenitis. There is no explanation why this extremely common lesion should heal in the majority of cases whereas in some 10 per cent. an erosion should become a chronic ulcer.

6. Inflammatory gastritis, identical with that found in association with peptic ulceration or occurring alone, was found in association with gastric cancer. No evidence was found to support the theory that inflammatory gastritis, or any sequel of it, can be regarded as a pre-cancerous state.

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INHIBITION OF VARIOUS CLOSTRIDIA BY PENICILLIN IN HUMAN SERUM

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(PLATES LXX AND LXXI)

THE work described in this paper was an attempt to find a basis for an adequate dosage of penicillin in the treatment of clostridial infections in man. For this purpose it was considered necessary to discover : (1) the concentrations of penicillin inhibitory to various strains of clostridia ; (2) a method of demonstrating, in human serum, concentrations of penicillin of the same order ; (3) the duration of an inhibitory concentration in the serum of man following single intramuscular doses of penicillin.

Five cultures of *Cl. welchii* from different sources were used, namely strains " T ", " E " and " V " of unknown origin, no. 273 of the National Collection of Type Cultures, and strain " R " from normal fæces. One strain each of *Cl. œdematiens* and *Cl. septique* was also used.

All strains of *Cl. welchii* produced typical colonies on blood plates anaerobically, broke up glucose agar in shakes and produced a stormy clot in litmus milk containing a piece of iron. They produced a vigorous Nagler reaction when grown on nutrient agar containing human serum (Hayward, 1943) and neutralisation by antitoxin occurred in every case. Each strain was virulent for guinea-pigs. Strain " T ", later found to be the most resistant to penicillin, kept its characteristics through repeated subcultures for 6 months.

Preliminary testing of Brewer's medium (Brewer, 1940) was carried out to exclude the inactivation of penicillin by the medium itself, and it was found that (a) titrations done aerobically with the Oxford strain of *Staph. aureus* (N.C.T.C. no. 6571) in nutrient broth and in Brewer's medium were practically identical, (b) titrations with *Cl. welchii* done aerobically in Brewer's medium and anaerobically in broth also agreed closely, and (c) the drop in potency, as measured by the cylinder test (Heatley, 1944), when a penicillin solution was mixed with an equal volume of sterile culture medium and incubated for 16 hours was exactly the same whether the medium was broth or Brewer's medium.

The concentration of penicillin inhibitory to various strains of clostridia

1. *In liquid culture.* A series of dilutions of a penicillin solution was made in 0·5 c.c. volumes in 15 cm. test-tubes. 14·5 c.c. of Brewer's medium were run into each tube from a pipette filled from a 100 c.c.

bottle previously inoculated with 0·25 c.c. of a 24-hour culture of the test Clostridium in Brewer's medium. (The tubes used for these experiments gave a fluid column about 8 cm. high with the quantities given.) The completed set of tubes was incubated for 16 hours at 37° C. At first, inhibition was judged by absence of turbidity of the medium, but it was later found convenient to add enough neutral red to the culture medium to give a final concentration of 1 in 10,000. The growth of an anaerobe in such a medium produces an intense greenish yellow fluorescence in sharp contrast to the original red colour, due to the intense drop in Eh produced by anaerobic growth.

Results.   Table I gives an average result of 31 experiments done with five of the strains tested. Strains "T", "E" and "V" of *Cl. welchii* all showed similar sensitivity. The table indicates that

TABLE I

Growth of clostridia in Brewer's medium containing penicillin

Final concentrations of penicillin (units per c.c.)	Growth of		
	Cl. welchii (strains T, E and V)	*Cl. septique* (1 strain)	*Cl. œdematiens* (1 strain)
0·6	—	—	—
0·4	∔	—	—
0·2	∔	—	—
0·1	+ +	±	±
0·0	+ + +	+ + +	+ + +

+ + + = growth showing a smooth turbidity throughout medium and froth at top (fig. 1, C).

∔∔ = less turbidity, part of medium clear (fig. 1, A).

∔ = still less turbidity, more than half medium clear.

± = a thin layer of turbidity either at the top or bottom of the tube, or discrete colonies (fig. 1, B).

— = no growth.

the highest concentration permitting growth was 0·4 unit per c.c., yet in individual experiments it ranged from 0·1 to 0·8 unit per c.c. Strains "R" and 273 were found to be more sensitive; in four experiments they were completely inhibited by 0·06 unit per c.c. The strains of *Cl. septique* and *Cl. œdematiens* were more sensitive than *welchii* strains "T", "E" and "V" but less so than "R" and 273. It was usually found that the control tube in dilution tests was turbid and frothing after two hours' incubation, but no change was visible in any of the tubes containing penicillin. Some of them would show growth after further incubation, but it was less vigorous than in the controls. In addition to this slowing of growth in concentrations too weak to cause total inhibition, penicillin produced growth abnormalities, particularly with strain "T" and to a lesser extent with strains "E" and "V". After 48 hours' incubation a

PLATE LXX

INHIBITION OF CLOSTRIDIA BY PENICILLIN

FIG. 1 —Cultures of *Cl welchii*, strain "T", grown in Brewer's medium (A) for 72 hours at 37° C without penicillin typical rods (B) for 48 hours at 37° C. in 0 4 unit of penicillin per c c filamentous forms (C) for 24 hours at 37° C in fresh Brewer's medium from abnormal colony in culture B , typical rods.

proportion of tubes in a titration developed objects macroscopically resembling mould colonies floating in a clear medium (fig. 1, B). Microscopically these colonies consisted of a tangle of threads imperfectly divided by occasional septa (fig. 3). When such a colony was implanted into fresh Brewer's medium, it recovered the cultural characteristics of the parent organism (figs. 1 and 4).

2. *On agar blocks.* Morphological changes produced by growing clostridia in small concentrations of penicillin were also studied by inoculating the spores of *Cl. septique* on to small blocks of nutrient agar containing 0·2 per cent. sodium thioglycollate and from 0·02 to 0·5 units of penicillin per c.c. These blocks after 10 hours' incubation at 37° C. were examined with the naked eye and under the microscope : the results are summarised in table II.

TABLE II

Morphological changes in Cl. septique *partially inhibited by penicillin*

Effect of penicillin on growth appearances of agar slab cultures			
Strength of penicillin (units per c c.)	Macroscopic	Microscopic	$\frac{\text{Length}}{\text{Breadth}}$ ratio
0·0	Numerous compact colonies	Spores and numerous short bacilli in well defined colonies	3 : 1
0·02	Numerous colonies less well defined than control	No spores: bacilli somewhat less closely arrayed ; longer	5 : 1
0·5	No colonies	Occasional shadowy bacilli	...

Inhibition of clostridia by human serum containing penicillin

Samples of human serum were made suitable for allowing clostridial growth by the addition of 1 : 500 sodium thioglycollate and then incubating in Wright's slide cells (Colebrook *et al.*, 1923) or the capillary tubes described by Fleming (1944). Penicillin titrations were done in duplicate in slide cells and capillary tubes, with and without sodium thioglycollate. This experiment was done repeatedly with staphylococci and with the " T " strain of *Cl. welchii*. No demonstrable difference in the level of inhibition could be attributed to the presence of the thioglycollate. (The growth of *Cl. welchii* in small volumes of serum in an anaerobic jar was found to be less reliable than that produced by the method described.)

The following method was then used for demonstrating the inhibition of clostridia by penicillin in serum. On a waxed slide were placed 5-c.mm. droplets of (*a*) saline as a control, and (*b*) solutions of penicillin in order of increasing concentration. With each droplet

were then mixed 45 c.mm. of the following mixture (hereafter described as " Mixture X ") :

450 c.mm. of fresh serum
50   .,   ,, 2 per cent. neutral sodium thioglycollate
25   ..   ,, a 1 : 100 dilution of a 24-hour culture of Clostridium in Brewer's medium.

The samples were then run into slide cells which were sealed and incubated overnight. In the control cell, *Cl. welchii* produced the following appearances : (*a*) compact discrete colonies, (*b*) a dense halo of turbidity around each colony (the Nagler reaction), (*c*) gas bubbles, and (*d*) hæmolysis of red blood corpuscles if any were present.

The strains of *Cl. œdematiens* and *Cl. septique* grew readily under these conditions, producing gas and hæmolysis; *Cl. œdematiens* produced discrete colonies, *Cl. septique* a diffuse haze.

The strains of *Cl. welchii* " T ", " E " and " V " were completely inhibited by 0·8 Oxford unit per c.c. and partly inhibited by concentrations down to 0·2 unit per c.c. These findings thus agreed with those obtained in Brewer's medium alone (see table I). Strains 273 and " R " were inhibited by about 0·06 unit per c.c., but whereas this was constant over a considerable range of inoculum size, the more resistant strains " T ", " E " and " V " gave an end-point dependent on the number of bacteria. There was little difference, however, when the number of organisms per cell was between 30 and 300.

The duration of an inhibitory concentration in the serum of man following single intramuscular doses of penicillin

This was investigated as follows. (*a*) Five c.c. of the subject's blood (a volunteer) were drawn from a vein and allowed to clot in a dry tube. (*b*) A single injection containing a known number of Oxford units of penicillin was given intramuscularly. (*c*) Small volumes of capillary blood were drawn off at intervals in Wright's capsules and allowed to clot. (*d*) The serum from each blood sample was collected and centrifuged to clear if necessary, and set up as follows. To each sample of serum, thioglycollate and *Cl. welchii* culture were added so as to form a mixture of the same proportions as " Mixture X.". With the exception of the control sample twofold dilutions were made, using the control mixture as diluent. Fifty c.mm. of each were then run into slide cells, sealed and incubated overnight. The finished slide cells contained almost undiluted test serum in the first chamber, and dilutions of 1 : 2, 1 : 4 and 1 : 8 in the succeeding chambers, with 30-300 organisms per chamber.

Experiments were done using (*a*) single intramuscular injections ranging between 15,000 and 100,000 units and (*b*) continuous drips of 100.000 units in 12 hours. None of the subjects was ill, the first

INHIBITION OF CLOSTRIDIA BY PENICILLIN

FIG. 2.—Strain "T". Photomicrograph of culture A, without penicillin, showing unaltered typical rods. ×1000.

FIG. 4.—Strain "T", after return to Brewer's medium (culture C), showing recovery of typical morphology. ×1000.

FIG. 3.—Strain "T", after growth in 0·4 unit of penicillin (culture B), showing filamentous forms. ×1000.

being a healthy man engaged in a sedentary occupation and the others convalescent wounded soldiers lying in bed. The inhibition of clostridia following single injections compared with that of the Oxford *Staph. aureus* is set out in table III. The sera of the two

TABLE III

Duration of serum inhibition for clostridia compared with the Oxford Staph. aureus following single intramuscular injections of penicillin

No. of expt	Subject of expt	Dose injected (units)	Organism used	Results at different times (minutes) after injection *						Duration of total inhibition of Cl. welchii	Condition of subject
				30	60	90	120	150	240		
1	A A	100,000 100,000	Staph Cl. welchii, strain T	1:8 1:8	1:4 1:2	1:4 ±	+ ∓	∓ ∓	∓ ∓	1½ hrs	Ambulatory
2	B B	100,000 100,000	Staph Cl. welchii, strain R	+ 1:8	+ 1:1		+ 1:1	+ 1:1	± −	3 hrs	In bed
3	C C	100,000 100,000	Staph Cl. welchii, strain R	+ 1:8	..	T 1:8	+ 1:2	+† 1:1†	∓	2½ hrs	In bed
4	D D	33,000 33,000	Staph Cl. welchii, strain T	+ 1:2	+ 1:4	T ±	+ ±	∓	∓	1 hr	In bed
5	B B	33,000 33,000	Staph Cl. welchii, strain R	+ 1:4	+	± 1:1	±	±	±	1½ hrs	In bed
6	C C	15,000 15,000	Staph Cl. welchii, strain R	+ 1:1	+ 1:2		+	∓	−	1 hr	In bed

* 1:8, 1:4, 1:2, 1:1 = highest dilution ...
+ =
± =
− = no inhibition
† 150 minutes after injection

men receiving continuous drips did not inhibit the "R" strain of *Cl. welchii* (the most sensitive of those tested), although both inhibited the Oxford *Staph. aureus*.

Discussion

It is known that penicillin inhibits pathogenic clostridia *in vitro* (Chain *et al.*, 1940). Its use in the treatment of experimental anaerobic infections in animals has been described (Chain *et al.*, 1940 ; McIntosh and Selbie, 1943 *a* and *b*; Hac, 1944 ; Nagler, 1945 ; etc.), and human infections have also been treated and the results reported. Jeffrey and Thomson (1944-45), with a dosage of 15,000 units 3-hourly in conjunction with approved surgical and serological methods of treatment of gas gangrene, were able to claim a mortality rate of 36 per cent. (33 cases), when the recognised rate up to that time was about 50 per cent. (MacLennan and Macfarlane, 1944). Cutler and Sandusky (1944-45) were not able to prevent the onset of gas gangrene in 4 cases treated prophylactically with 10,000 units 3-hourly.

Both therapeutic and prophylactic results were better when larger doses were used. Thus Langley and Winkelstein (1945) report the routine use in 96 cases of 40,000 units 4-hourly or 20,000 units 2-hourly in combination with sulphonamides, and a fatality rate of 11·5 per cent.

Gledhill (1945) reported recovery, though several amputations were necessary, in all but one instance in 33 cases of gas gangrene treated by irrigation of wounds with 500 units per c.c. in combination with 20,000 units intramuscularly 3-hourly. After D day, when the use of 100,000 units followed by 45,000 to 50,000 4-5-hourly or 20,000 3-hourly was recommended prophylactically, Fisher *et al.* (1945) were able to claim that no deaths or amputations for gas gangrene or any allied condition had occurred in 4000 consecutive battle casualties treated at a transit hospital in the earliest weeks of the invasion. The prevailing incidence of gas gangrene alone for the B.L.A. was 0·27 per cent., with a mortality of 20 per cent. (Porritt *et al.*, 1945).

In the five random strains of *Cl. welchii* tested, considerable variation in sensitivity to penicillin was demonstrated. The cultures of *Cl. œdematiens* and *Cl. septique* tested were of intermediate sensitivity. The range of in-vitro resistance of the group as a whole was 2-30 times that of the Oxford *Staph. aureus*. Yet partial inhibition and atypical morphological forms were demonstrable with the most resistant strains in concentrations of penicillin only 10 times that necessary to inhibit the standard staphylococcus. Thus the in-vitro production of abnormal and filamentous forms in weak penicillin has already been described by Gardner (1940) and demonstrated with *Cl. welchii* by Fleming (1945), and a similar phenomenon has been produced by Nagler in the muscles of guinea-pigs experimentally infected with *Cl. welchii* and convalescent after continued penicillin and antitoxin therapy.

Though these in-vitro experiments would seem to indicate that a dosage for clostridial infections might need to be 10 times that for staphylococcal infections, yet the work with human volunteers, although incomplete, suggests that a dose of no more than 100,000 Oxford units of penicillin should maintain even an apyretic patient's serum completely bacteriostatic to different strains of *Cl. welchii* for 1½-3 hours. A dose of only 33,000 units produced complete inhibition of the most resistant strain for 1 hour, while one as low as 15,000 produced the same effect on one of the most sensitive strains. In toxæmic patients whose excretory rate is lower, complete inhibition may be more prolonged and favourable therapeutic action may well continue longer still, in fact as long as the serum penicillin is sufficient to produce partial inhibition as demonstrated by the formation of filaments. These results seem therefore to offer a rational explanation of the partial response to penicillin therapy shown by Jeffrey and Thomson's series, and of the improvement obtained when higher concentrations were used, either by larger intramuscular doses or by local instillation, as shown by the results of Fisher *et al.* and of Gledhill.

Summary

1 An adaptation of Wright's slide cell method for the demonstration of clostridial growth in human serum and its inhibition by penicillin is described

2 With this technique 5 strains of *Cl welchii* were found to be between 2 and 30 times as resistant to complete bacteriostasis by penicillin as the Oxford staphylococcus

3 However, by the same technique it was shown that 100,000 units of penicillin injected intramusoularly maintained a concentration of penicillin in the serum of apyretic persons fully bacteriostatic to these strains for $1\frac{1}{2}$ 3 hours, and that lower doses were effective for shorter periods

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THE ABSORPTION OF WAR GASES BY THE NOSE

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From the Experimental Station, Porton

(PLATES LXXII-LXXIV)

WHEN animals inhale lethal concentrations of mustard gas [S(CH₂CH₂Cl)₂] or nitrogen-mustard [CH₃N(CH₂CH₂Cl)₂] vapour, death results from direct damage to the respiratory tract with or without systemic poisoning. With certain small species, however (rabbit, guinea-pig and rat), death from systemic absorption is frequently observed with little or no damage in the respiratory tract,[*] apart from severe inflammation of the nose, which is always present. In contrast, exposure to phosgene usually results in death whenever certain concentrations and times of exposure are exceeded, and all species of animals show pulmonary lesions. It appears, therefore, that a lethal dose of certain vapours may be absorbed through the mucous membrane of the nose The present paper describes an experimental investigation of this hypothesis

METHOD

Most of the experiments were carried out on rabbits. The complexity of the nasal skeleton in this species made it desirable to repeat the experiments on a species more closely resembling man, and for this purpose the monkey (*Macacus rhesus*) was used in four experiments Rabbits were anæsthetised with nembutal, the trachea divided and cannulæ inserted into the cut ends (fig. 1) The animal was placed inside a one cubic metre chamber and the lower cannula, which served to aerate the lungs, was connected to the outside air. This tube was kept as short as possible in order to avoid unnecessary dead space The upper cannula receiving air from the nose was connected to a bubbler outside the chamber through which a measured quantity of air was sucked by water displacement at the rate of one litre/min. for 10 minutes. This is a fair approximation to the respiratory volume of a normal rabbit. Samples of air from the chamber were obtained by suction through a second bubbler at the same rate and for the same time

Nominal concentrations of 40, 100 and 500 mg /m³ of the gas under investigation were put up in the chamber and the actual concentrations and percentages recovered from the nose were calculated from the amounts found in the two bubblers. So far as possible experiments with the three increasing concentrations were performed on the same rabbit. Sometimes, however, much condensation of water vapour occurred in the nasal sampling tube or the nasal

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[*] The late Professor A E Boycott also noted this during the 1914 1918 War, but offered no explanation

passages became blocked with mucus. When this happened the results were ignored. In all the experiments recorded the air was flowing freely. In most experiments the rabbits' mouths were sealed with Michel's clips in order to
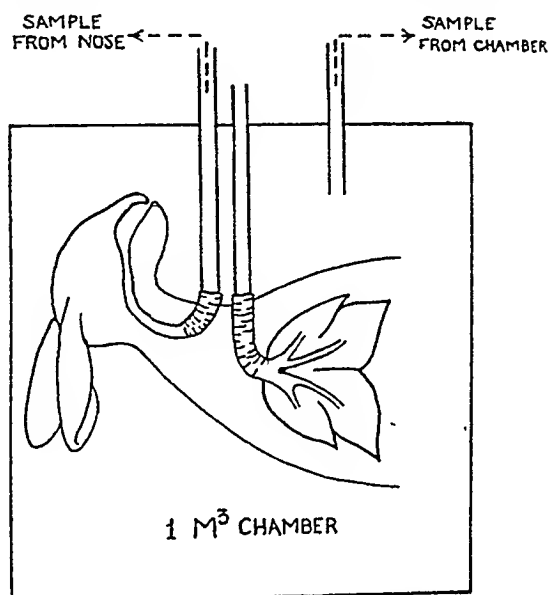
FIG. 1.—Diagram showing method of sampling.

limit suction to the nose. In experiments with monkeys, sampling was carried out at the rate of half-a-litre/min., approximating to the respiratory volume of that animal. No difficulty arose in keeping up a uniform flow whilst sampling and there was no interference from excessive nasal secretion. The other procedures were similar to those in the rabbit experiments.

The animals were killed at the conclusion of the experiments and the
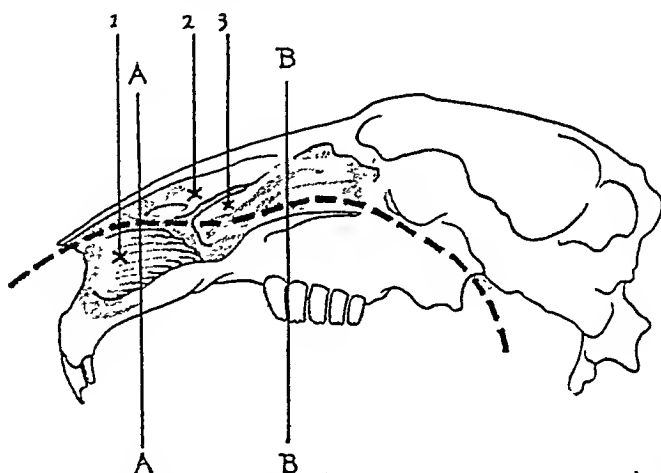
FIG. 2.—Diagram of sagittal section of rabbit's nose, showing the two levels of transection at A and B. (1) Maxillo-turbinals. (2) Naso-turbinals. (3) Ethmoturbinals. The dotted line shows the direction of the air current.

rabbits' heads removed, fixed in formol-alcohol and decalcified in 10 per cent. nitric acid. The nose was transected at two levels (fig. 2) and paraffin sections

were stained with Ehrlich's acid hæmatoxylin and eosin, van Gieson's stain and Heidenhain's iron hæmatoxylin The heads of normal rabbits were treated similarly

In order to follow the time sequence of nasal changes after exposure to various concentrations of the gases, we studied a large additional series of animals killed at short intervals up to 3 days Fixation, decalcification and staining were similar to the above

RESULTS

The amounts of gas recovered from the nose, expressed as a percentage of the chamber concentration, are given in the table The results show reasonable regularity and probably justify the assumption that the percentage of gas removed by the nose is independent of the chamber concentration and therefore constant for a given gas It will be seen that about 75 per cent of phosgene can be recovered after passage through the nose of the rabbit With mustard vapour, however, only 20 per cent can be accounted for, and with nitrogen mustard vapour no more than 10 per cent In monkeys, the contrast is less striking, owing possibly to their less complicated nasal structure

Structure of the nose of the normal rabbit

In the normal rabbit the nostrils are two small openings, admitting a probe of not more than 6 mm diameter The nasal cavities are separated by a bony septum which is expanded anteriorly by the organ of Jacobson and posteriorly is incomplete Both cavities contain three types of turbinate bones Anteriorly are the maxillo turbinals, which form a complicated lamellar structure The less complicated ethmo turbinals are situated in the posterior portion, whilst above are the simple naso turbinals consisting of single bony lamellæ (figs 2 4) These structures consist of a mucous membrane with blood vessels, lymphoid tissue and glands of Bowman covered by epithelium and lying upon a delicate bony framework The direction of the air current, dorsal to the maxillo turbinals and ventral to the ethmo turbinals, is shown in fig 2

The epithelium is of the columnar ciliate type and below it is found a profuse vascular plexus of very regular pattern (Swindle, 1935), associated with the glands of Bowman and, particularly in the posterior portion of the nose, with lymphoid tissue, which is also abundant in the floor of the nose in both anterior and posterior sections

The lymphatic drainage of the nose empties chiefly into the lymphatic trunk accompanying each internal jugular vein A few lymphatics accompany the facial vein, emptying into the trunk which runs parallel with the external jugular vein A third group joins the posterior pharyngeal plexus and empties mainly into the duct of the same side, above the lowest lymph node lying beside the internal jugular vein

. *Pathological changes produced by mustard gas and phosgene*

Both mustard gas and nitrogen-mustard gas induce a rapid developing inflammation of the nasal cavities, associated with hæmorrhage and necrosis of lymphoid tissue. These changes closely resemble

TABLE

Absorption of war gases by the nose in rabbits and monkeys

		Rabbits				Monkeys		
	Expt. no.	Chamber concentration mg./m³	Nose concentration mg./m³	Percentage recovered	Expt. no.	Chamber concentration mg./m³	Nose concentration mg./m³	Percentage recovered
Phosgene	1	184 350	140 290	76 83				
	2	107 860	98 630	92 73	1	90·5 390 2020	48 105 1940	53 27 96
	3	49·5 126	38 96	77 76	2	224 1152 2605	51 1126 2500	23 98 96
	4	38 124 392	27 80 332	71 64 85				
	5	48 56 258	28 37 243	58 66 94				
	6	39·5 155 476	38 145 455	96 93 95				
Mustard gas	1	33 60 195	5·5 18 45	17 30 23				
	2	26·3 60 225	4·5 12 24·5	17 20 11	1	86 133 205	9·1 14·4 72	10 11 35
	3	15 36 120	1·5 3·2 12	10 9 10	2	61·5 226 350	5·3 70 67	9 31 19
	4	38 78 125	3·2 12·2 22·4	8 16 18				
Nitrogen-mustard	1	20 81 400	3·9 12·5 44·5	19 15 11				
	2	53·8 160	5·9 16·7	11 10				
	3	36·7 127	2·3 5·5	6 4				

the membranous tracheitis and bronchitis found after inhalation the vapour. The anterior portion of the nose shows greater dam

PLATE LXXII

ABSORPTION OF WAR GASES BY NOSE
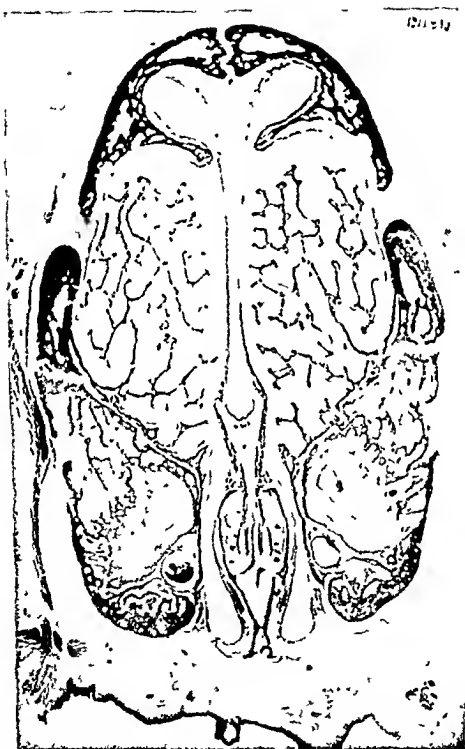
FIG 3.—Coronal section of a rabbit's nose at level A (fig 2) The complicated lamellæ of the maxillo turbinals are shown filling the nasal cavity Heidenhain's iron-hæmatoxylin ×5.

PLATE LXXIII

ABSORPTION OF WAR GASES BY NOSE

FIG 4 —Coronal section of a rabbit's nose at level B (fig. 2). The simple lamellæ of the naso-turbinals project above, whilst the ethmo turbinals project into the nasal cavity from the lateral walls. Heidenhain's iron hæmatoxylin. × 5.

than the posterior, especially towards the dorsum and therefore nearest to the air-stream.

In severe cases a large blood clot may occupy most of the nasal passages. In less severe cases this is most marked in the dorsal portion of the maxillo-turbinals, where adjacent lamellæ may be welded together by a solid mass of blood clot with leucocytes (figs. 5 and 6). A similar appearance is seen in the naso-turbinals and less frequently in the ethmo-turbinals. In milder cases, damage is found only in the anterior turbinate bones and consists of small hæmorrhages in congested mucous membrane, with polymorphonuclear infiltration.

It is remarkable how consistently the glands of Bowman and organ of Jacobson escape injury. Yet nasal lymphoid tissue damage is considerable, particularly in the anterior portion of the nose. After 12 hours damage is also found in the cervical lymph nodes which drain the nose and indeed in lymphoid tissue everywhere. Lymphocyte nuclei are pyknotic or unrecognisable, and infiltrating histiocytes contain much pigment which has been identified with "Abnutzungs-pigment" of Lubarsch.

With phosgene, changes in the nose are very slight—at most, a mild congestion.

DISCUSSION

It appears from these experiments that 80-90 per cent. of mustard gas or nitrogen-mustard gas is lost during passage through the nose. With phosgene only about 25 per cent. is lost. Severe pathological changes are produced by the mustard gases but phosgene gives barely recognisable changes. Mustard vapours often enough have little effect on the lungs but phosgene seldom if ever fails to produce pulmonary œdema. Nevertheless mustard gases may produce lethal effects in the absence of lung damage. We suggest that our original hypothesis is supported by these observations and that the nose, while it may help to protect the lungs against direct damage by the mustard vapours, may itself suffer much damage and act as the route whereby a lethal dose of these vapours is absorbed into the body.

The route of absorption of the mustard gases still remains undecided. Our experiments do not tell us whether damage to nasal lymphoid tissue is the result of direct local action or of systemic absorption. In the sampling experiments, damage to the cervical lymph nodes draining the nasal mucosa was not found, since the animals were not allowed to survive. In the experiments where the time sequence was studied, damage to the cervical lymph nodes cannot be attributed solely to lymphatic absorption from the nasal mucosa, since identical changes result from the skin application or subcutaneous injection of mustard gas. With these latter routes of administration damage must be the result of systemic absorption by which lymphoid tissue everywhere is affected. We are unable to say whether damage to the cervical lymph nodes precedes or coincides

with damage to lymphoid tissue elsewhere. Histological examination, moreover, has not made it possible to distinguish in lymphoid tissue the effects of absorption by either route.

The fact that mustard gas was removed more completely than phosgene from the air by the nose may seem surprising. For example, the following data are given by Sartori (1939). The solubility of phosgene in water is difficult to measure owing to its rapid hydrolysis, but when 1 g. of phosgene was shaken with 100 c.c. of water at 0° C. it was dissolved and completely decomposed in barely 20 seconds (Sartori, p. 67). The solubility of mustard gas in water at 25° is only about 0·069 per cent. by weight (Sartori, p. 225). Since the mucous membrane of the nose is covered by a watery solution, it might therefore be expected that phosgene would be removed from the air more completely than mustard gas.

This view of the matter neglects the fact that phosgene is much more volatile than mustard gas. At 20° C. the vapour pressure of phosgene is about 1173 mm. Hg. (Sartori, p. 65), while that of mustard gas is only 0·115 mm. Hg. (Sartori, p. 224). The concentrations of the two gases used here were of the same order, but the percentage saturation of the air with mustard gas must have been much greater than the percentage saturation with phosgene. This difference would explain the comparatively greater uptake of mustard gas, but other factors may also play a part. The total amount of mustard gas removed from the air in the third part of expt. 2 (rabbits) was about 2 mg., and about 3 c.c. of water would be needed to dissolve this quantity. It seems likely, therefore, that mustard gas rapidly disappeared from the surface of the mucous membrane during exposure. The hydrolysis of phosgene at the beginning of the experiment, on the other hand, would liberate acid products which might make the surface acid and so inhibit the uptake of more phosgene. The facts are thus less surprising than appears at first sight. The physical properties of nitrogen-mustard are similar to those of mustard gas, but it is rather more soluble in water and more rapidly hydrolysed. Those facts would explain its slightly greater uptake in the nose.

SUMMARY

1. In rabbits and monkeys a considerable proportion of the vapour of mustard gas and nitrogen-mustard gas is lost during passage through the nose, where severe inflammatory changes are produced.

2. Phosgene passes through the nose without much reduction in its concentration and only slight nasal congestion is found.

3. These facts are correlated with the striking absence of lung damage in small animals dying after exposure to the vapour of mustard or nitrogen-mustard and are contrasted with the constant occurrence of pulmonary œdema following exposure to phosgene.

ABSORPTION OF WAR GASES BY NOSE
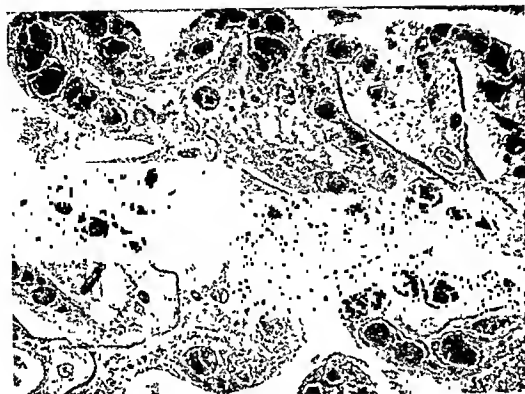
FIG. 5

FIG. 6

FIGS. 5 and 6.—Mucous membrane of the maxillo-turbinals, showing severe damage following exposure to the vapour of mustard gas. Hæmorrhages are present in the mucosa and all vessels are intensely congested. Exudate and blood clot fill the interstices of the lamellæ. **Ehrlich's acid** hæmatoxylin and eosin.   × 50.

4. It is suggested that the nose may be an important route of absorption for the vapour of mustard and nitrogen-mustard gas.

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SWINDLE, P. F. . . . . . . 1935. *Ann. Otol. Rhinol. and Laryngol.,* xliv, 913.

LOWER ACCESSORY PULMONARY ARTERY WITH INTRALOBAR SEQUESTRATION OF LUNG: A REPORT OF SEVEN CASES

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(PLATE LXXV)

THE seven cases presented in this paper are examples of a congenital abnormality which has been adequately described only once before (unpublished Düsseldorf thesis by Fischer, quoted by Muller, 1928). There are two other cases in the literature but these are only shortly referred to in the report of a meeting of the American Society of Thoracic Surgeons (Haight, 1941-42). The abnormality consists of a large artery to the base of the lung from the nearby aorta, and a congenital bronchial dislocation in the part supplied.

The bronchial abnormality in each of the present cases took the form of a bronchopulmonary mass or cyst which, although included within the lower lobe, was dissociated from the normal bronchial tree. There were adhesions which may have been primary, but the artery entered the lung in the line where the pulmonary ligament should run and the mass or cyst was constantly situated in this part of the lower lobe. In some cases the mass was composed of normally differentiated but bronchiectatic and inflamed lung tissue. In other cases there was a large cyst which was obviously bronchial. In one case the mass was polycystic, whilst in another polycystic disease was present elsewhere in the lung. The arterial abnormality was not related to any cardiac malformation and was purely accessory. The exact origin of the vessel in some operation cases was uncertain, but it appeared in all to have arisen from the aorta in the vicinity of the diaphragm. In Haight's two cases it probably came from the upper part of the abdominal aorta; in Fischer's case and in at least four of the present cases from the lower part of the thoracic aorta.

The various forms which the abnormality may take are shown in figs. 5-8. In Fischer's original case (fig. 6) branches of the abnormal artery entered the adjacent normal lung, whilst some branches of the normal artery were also stated to have entered the mass. The latter occurrence was not seen in any of the present cases, but in two of them, branches of the abnormal artery supplied normally connected lung. The posterior basal sector in cases II and VII were thus

supplied. There have been a considerable number of cases in which a similar artery was present, but in which the bronchial abnormality was not described (Huber, 1777; Maugars, 1802; Meckel, 1820; Hyrtl, 1839; Eppinger and Schauenstein, 1902; McCotter, 1910; Park, 1912-13; Batts, 1938-39; Harris and Lewis, 1939-40). Menke (1936) described an accessory artery which arose from the right subclavian. It is quite possible in some of these cases that the bronchial abnormality was overlooked. Indeed in some (*e.g.* Park) the lung was not incised. In others (*e.g.* McCotter) bronchial displacement was undoubtedly absent. The abnormality, as shown in figs. 5-8, therefore presents itself in three ways: type I, in which the artery goes to normally connected lung; type II, in which it is distributed to the sequestrated mass and to the adjacent lung; and type III, in which it is confined to the mass. The bronchial abnormality is rather more frequent on the left side, Fischer's case and four of the present seven cases being on this side. In Haight's two cases the side is not stated. Cases in which a lower accessory artery was reported by itself however were more common on the right. Of these, five were on the right, three on the left and one was bilateral.

The susceptibility of these abnormal arteries to atheromatous degeneration was noted by Batts. Degenerative changes were more marked in the older subjects, but there was also a close correlation with sepsis. Thus atheroma was slight in cases II (fig. 1) and III (aged 7 and 12) in whom inflammatory changes were slight; but marked in case IV (aged 9) in whom inflammatory changes were marked (fig. 2). This is probably the result of damage to the vessel wall by diffusible inflammatory products. The bronchopulmonary abnormality predisposes to post-pneumonic sepsis and these cases tend nowadays to fall into the hands of the thoracic surgeon. The incidence of the condition among 280 pulmonary excisions was 1·7 per cent.; five cases came from the Thoracic Surgical Unit, Harefield Sanatorium, the other two from the London Chest Hospital and the Royal Infirmary, Cardiff. They are presented in the order of their reception.

Case reports

Case I. Man of 33 with 21 years' history of pneumonic attacks. Sputum increased and vital capacity reduced. Condition poor, with some wasting but no clubbing of fingers. The abnormal artery was not seen at operation so that its exact origin is uncertain.

Right pneumonectomy (fig. 8) showed polycystic disease of upper, middle and apical portions of lower lobes. A very atheromatous artery (0·45 cm. diameter) entered at the lower border and supplied a chronically inflamed pale yellow mass in the posterior medial part of the lobe between the hilum and the base. In this there was a dilated flattened bronchus-like structure lined with respiratory epithelium but containing no muscle, cartilage or glands. It began blind near the accessory hilum, branched in the same direction as the artery, but had no connection with the bronchial tree. The venous drainage was to the normal pulmonary veins.

PLATE LXXV

LOWER ACCESSORY PULMONARY ARTERY

Fig 1.—Abnormal artery in case II to show elastic structure.   ×60.

Fig 2.—Atheromatous artery in case IV.   ×35

Case II. Half-casto girl of 7 with a history of three pneumonic attacks. X-ray showed a basal cyst. At operation a large artery was seen to come from behind the pericardium but its origin from the aorta was not reached.

Right lower lobectomy specimen (figs. 3 and 4), with an abnormal artery (0·4 cm. diameter), which entered a firm mass in the posterior medial part of the
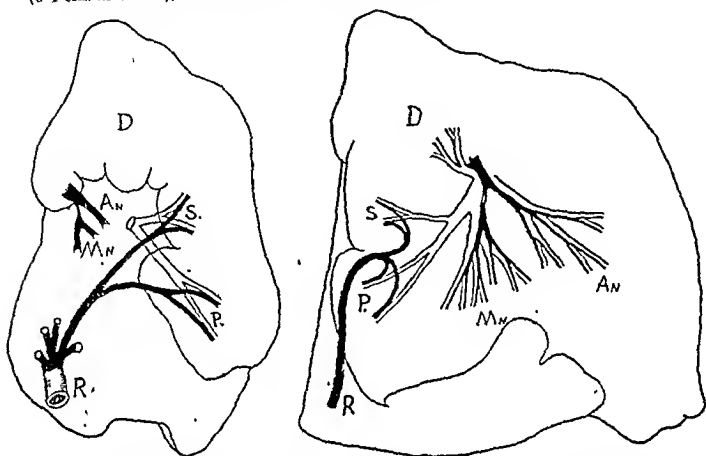
FIGS. 3 and 4.—Intralobar sequestration (shaded) in case II. Stenosis of normal artery to mid-basal sector, and "pulling away" of abnormal artery to subdorsal and posterior basal sectors.

D = dorsal sector.
P = posterior basal sector.
S = subdorsal part of posterior basal sector.
An = normal artery to anterior basal sector.
Mn = artery to mid-basal sector (stenosed).
R = accessory pulmonary artery.

base. On section this was polycystic and was separated from the rest of the lobe by a large distension bulla. The bulla was aerated by valvular slits in its roof, whilst its floor freely communicated with the polycystic mass. The spaces in the mass had a respiratory epithelial lining but no cartilage or glands. They were separated by a collagenous stroma in which were rudimentary alveoli. Embedded in the mass was a fully formed bronchus which began blind near the accessory hilum alongside the artery and branched into the spaces of the mass. The mass was supplied exclusively by the abnormal artery. In this case, however (as in case VII), the abnormal artery also supplied adjacent normal lung. The subdorsal and posterior basal sectors were exclusively supplied in this way. The texture of the lung with this abnormal supply was normal. The branch of the normal pulmonary artery to the mid-basal sector was stenosed. The venous return was normal.

Case III. Boy scout of 12 with recent hæmoptysis and pain in the chest, who was found to have pulmonary cysts. At operation the abnormal artery was seen to arise from the lower part of the thoracic aorta.

Left lower lobectomy specimen (fig. 7) with 4 branches of accessory artery (0·3, 0·3, 0·1 and 0·1 cm. diameter) which entered at the lower end of the aortic

groove. In the part supplied by the abnormal artery there was a system of branching bronchi which began blindly at the accessory hilum. These were bronchiectatic and the related parenchyma was chronically inflamed. There was close correspondence between the branching of the abnormal artery and the bronchus. As in the preceding case the dissociation was incomplete. There were two groups of aerated cysts intervening between the mass and the rest of the lobe, which communicated with branches of both the normally connected and the dissociated bronchial trees. Apart from these cysts, which had a ciliated epithelial lining, the demarcation of the mass was not sharp. The venous drainage was normal.

Case IV. Girl of 9 with a 2 years' history beginning with a febrile illness. There was left basal dullness, with no filling of this part on repeated broncho-graphy although filling in adjacent parts was good. Many operations were performed for empyema and abscess. At the operation for lobectomy the abnormal artery was seen to arise directly from the aorta.

Left lower lobectomy specimen showed a white fibrous mass in the posterior third of the base. This contained a branching system of fully formed but chronically ectasic bronchi which had no connection with the normally connected bronchial tree. The abnormal artery entered on the posterior medial aspect of the mass by two branches (0·3 and 0·6 cm. diameter). The correspondence between the branching of the artery and the bronchus was close. The venous drainage was normal. A chronic abscess in the lateral part of the mass communicated with the dissociated bronchial tree by several patulous bronchi. Instillation of opaque oil into the abscess cavity at a previous operation led to its complete filling. X-rays of this case have been previously published (Blair et al., 1946).

Case V. Sailor of 26 with a basal shadow which might have been a benign growth. History of pain 8 years ago and of fever 5 months ago. At operation an abnormal artery was seen to arise from the aorta and enter the lower lobe, which contained a pulsating cyst.

Left lower lobectomy specimen with a wedge-shaped mass in the posterior medial part of the lobe containing a large cyst of 4·5 cm. diameter. This resulted from gross mucous distension of an aberrant bronchus. In the specimen the abnormal artery had divided into two branches (0·5 and 0·4 cm. diameter) which entered about 2 cm. above the base. The larger branch was injected and stereoscopic X-ray photographs taken. The cyst was partly divided by an enormous carina and the larger chamber gave off 3 branches which corre-sponded with 3 branches of the larger artery. The surrounding parenchyma of the mass was atelectatic and inflamed, but in one part which projected into the adjacent normal lung it was finely polycystic. This polycystic snout contained a conspicuous cartilaginous element measuring 0·45×0·15 cm. The posterior basal bronchus was very small. The venous drainage was to the normal pulmonary veins.

Case VI. Boy of 16 with a basal cyst which had been previously treated as an empyema. Twice, when it was drained, there had been hæmorrhage by both tube and trachea. At operation a large elastic artery was seen running from the aorta just above the diaphragm and entering the base of the lung.

Left lower lobectomy specimen, occupied by a large cyst (10×9×5 cm.) which had no demonstrable communication with the basal bronchi. The normally connected lung was greatly reduced and confined to the anterior border. The cyst had a surgical hole in its outer wall. It had a lining of respiratory epithelium and cartilage and glands were present. The inner surface was trabeculated. As the result of previous ulceration some of the trabeculæ were ruptured. The structure may originally have been similar to the preceding case. Adjacent to the cyst was a layer of chronically inflamed lung about 0·3 cm. thick. A large abnormal artery (0·5 cm. diameter) entered
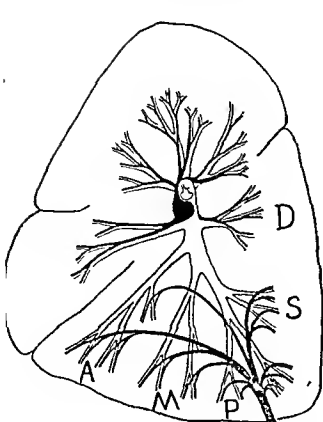
Fig. 5.—Abnormal artery without sequestration (type I).
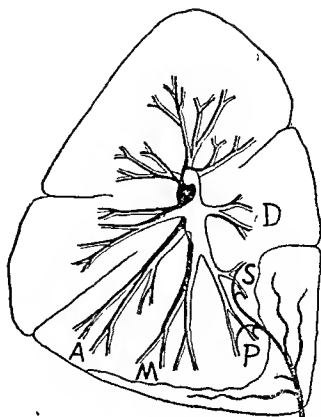
Fig. 6.—Abnormal artery to mass and adjacent lung (type II).
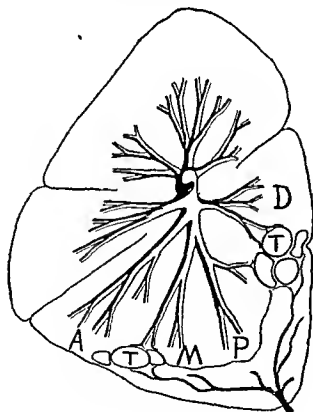
Fig. 7.—Type III, with intervening traction cysts (case III).
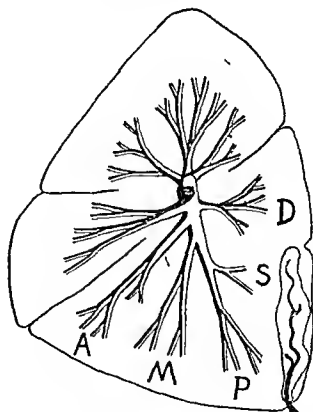
Fig 8.—Case I. Abnormal artery confined to sequestrated mass (type III).

A = anterior basal sector.
M = mid-basal sector.
P = posterior basal sector.
D = dorsal sector.
S = subdorsal part of posterior basal sector.
T = traction cysts (fig. 7)

the base just behind the infra-hilar notch and branched in the wall of the cyst. The veins ran to the normal pulmonary veins. An artery accompanying a lateral branch of the presumptive posterior basal bronchus was stenosed.

Case VII. A man of 59 with a 5 years' history of cough and sputum due to lung abscess following osteomyelitis of the femur. At operation a large artery was seen coming from behind the pericardium in relationship to an appendage of the lower lobe which extended into the mediastinum.

Right lower lobectomy specimen, shrunken and rough from adhesions. The abnormal artery (0·3 cm. diameter) was atherematous and calcified. It entered at the posterior medial end of the lower border, where there were several lymph glands. The mass was situated below the hilum in the posterior medial part of the base. It was fibrosed and white on section and contained little carbon pigment. Numerous epithelialised abscess cavities were present in all parts of the lobe, including the mass. These intercommunicated and in the parts outside the mass communicated with bronchi. As a result of the previous sepsis it was impossible to discern any definite branching system of bronchi in the mass itself. The bronchi in the normally connected lung were chronically inflamed and somewhat ectasic. They were accompanied by branches of the normal pulmonary artery except in the case of the posterior basal bronchus, which for nearly 3 cm. was unaccompanied and ran into a cavity in a part of the lung supplied by the abnormal artery. All veins found drained into the normal pulmonary vein.

Nature of the abnormal artery

Although arising from the aorta, most authors agree in regarding these abnormal arteries as pulmonary rather than bronchial, but their elastic nature (characteristic of pulmonary arteries) has not been previously noted. The abnormal artery in each of the present cases was elastic, but differed from the normal pulmonary in two respects : (1) being subject to systemic pressure it was thicker walled; (2) since it was carrying oxygenated blood, an accompanying bronchial artery was absent. It may be regarded as pulmonary and bronchial artery combined, but it is simpler to call it a systemic pulmonary. A pulmonary artery may be defined as the artery which provides the primary blood supply of developing lung tissue. In this respect the root connections are of secondary importance and may be normal or abnormal. If such a vessel supplies the rich capillary bed of developing lung tissue, it becomes of large size and elastic structure. The bronchial arteries on the other hand are small and muscular. But even when enlarged, as in the cases of pulmonary atresia with closed ductus described by East and Barnard (1938), they remain muscular. They arise with the segmental arteries and, although observation is lacking, they appear to be a secondary development dependent on the low oxygen tension in the normal pulmonary artery after the heart has divided into right and left halves.

It seems that the important distinction between muscular and elastic arteries is more familiar to pathologists than to normal anatomists and physiologists. The systemic arterial tree in man is elastic only to the ends of the common carotids, subclavians, and abdominal aorta. Branches beyond this, like the coronaries and

renals, are muscular   The elastic nature of the large proximal arteries facilitates the reception of the cardiac output   The capacity of the pulmonary circulation is small but accommodates the same extra volume with each beat   This however only partly explains why the pulmonary arterial tree is elastic throughout, since it does not account for the elastic nature of systemic pulmonary arteries   It is probable that the great vascularity of pulmonary tissue (leading to increased flow with low pressure and deficient vasomotor control) is a more important factor   If this is correct there should be a difference between the artery in the two cases of lower accessory lung described by Davies and Gunz (1944)   In case I it should be muscular, because there was no formation of alveoli , in case II it should be elastic, because the pulmonary structure was almost normal   In two recent cases of lower accessory lung with alveolar differentiation the artery was elastic

Systemic pulmonary arteries arise as the result of successful competition with the normal pulmonary artery   Harris and Lewis point out that they develop like the normal pulmonary artery in the vascular plexus enveloping the fore gut   This plexus has connections with both dorsal and ventral aorta, but the abnormal artery is rare because the normal embryonic shifts disrupt the dorsal connections   Anything which delays (or temporarily arrests) shift would promote their development   When, in spite of normal shifts, the dorsal aorta " captures " a bulbous tip of the embryonic bronchial tree, the subsequent development of the abnormal artery is usually associated with traction

The earlier these abnormal vessels begin the greater is the extent of their distribution in the lung, and by referring to the embryonic bronchial tree at different stages it is possible to date their origin   Thus the artery in Bencke's case II (1905) supplies the two sequestrated lungs and arises at 3 mm   In Klebs's case (1874 , quoted by Gruenfeld and Gray, 1941) it supplies the sequestrated right lung and arises at 5 mm   In Hyrtl's case it supplies the whole of the left lower lobe and arises about 8 mm   In Eppinger's case it supplies more and arises earlier, whilst in McCotter's case it supplies less and arises later   In cases II and VII it supplies the posterior basal sector and arises about the 10 14 mm stage   So far there has been no case with a distribution smaller than this   The minute arteries in the pulmonary ligament, which may be regarded as anatomical frustrations, have only a pleural distribution

Nature of the bronchopulmonary mass

The cases reported are really examples of sequestrated or ectopic lung   Ectopic pulmonary masses, quite distinct from the normally connected lungs, occasionally occur   They are most common in the lower part of the thorax or upper part of the abdomen, and are

usually referred to as lower accessory lungs. The present cases and those of Haight and Fischer show abnormalities of the same kind, in which however the sequestrated mass is included within the lower lobe. Müller has pointed out that there is an intermediate condition with two pedicles (an arterial attached to the parietes and a venous attached to the lung) in which the blind bronchus of the mass is situated at the arterial pedicle. There are of course other differences between intra- and extralobar sequestration :—

1. Extralobar sequestration shows a very marked left preponderance, only three of the forty or so human cases having been on the right side. Cases of intralobar sequestration are more evenly divided, three of the present series of seven having been on the right.

2. The venous drainage with extralobar sequestration is usually to the hemiazygos, with intralobar sequestration to the normal pulmonary vein. (In Park's case the right lung was supplied by an enlarged diaphragmatic artery, whilst the venous drainage was to the inferior vena cava, but it is not known whether sequestration was present.)

3. There is a well known association between extralobar sequestration and diaphragmatic hernia, which has not been noted with the intralobar type.

4. Infection is common with intralobar and uncommon with extralobar sequestration.

Pulmonary sequestration also occurs on a larger scale. In a case described by Klebs the right lung was not connected with the trachea but began as a blind bronchus attached to the lower part of the œsophagus. In a still more extraordinary case (Beneke, case II) the tracheal bifurcation was separated from the larynx during development, so that whilst the former was in its usual place the two bronchi communicated with the lower part of the œsophagus.

These various degrees of sequestration are all explicable on the basis of detachment of a bulbous tip of the bronchial tree at various stages in its development. Thus Beneke's case II would result from detachment of the initial bulbous tip which appears at 3 mm. The actual detachment might be later than this but it occurred in association with, and probably as a result of the acquisition of an abnormal blood supply which originated at 3 mm. The low attachment to the œsophagus indicates that the delay could not have been long, and the communication with the œsophagus limits it to the 5 mm. stage, by which time the trachea is tubulated. Similarly complete unilateral sequestration (Klebs) corresponds with detachment of the bulbous tip for the right lung, which appears at 5 mm. Intrinsic evidence in two of the present cases (II and VII) points to intralobar sequestration occurring about the 10-14 mm. stage. The increased left preponderance of extralobar sequestration suggests a later origin still, when the expanding pleural sacs envelop the pericardium, which by this time has become asymmetrical. The case of lower accessory

lung described by Rusby and Sellors (1944-45), in which there was a defect in the pericardium, provides definite evidence in support of this. Had the pericardial defect arisen before the pleural sacs expanded the pericardium would have communicated with the exterior. (The defect was not of course a persistence of the venous iter of Lockwood.)

It is uncertain if there is a deficiency of the normally connected bronchial tree in these cases attributable to the detachment. In some cases the posterior basal sector was small, but this might be due to the space occupied by the mass, and in one case in which the mass was very large (case VI) all three basal sectors were reduced in size. Vogel (1899) thought he detected a deficiency in the bronchial tree in two cases of lower accessory lung, but this is discredited. The possible absence of defect is explicable on the basis of Flint's work (1906-07) on the capacity of other bronchial tips to "take over". In complete sequestration, however, compensation is impossible and here agenesia is obvious and complementary.

Evidence of traction

This is best exemplified in case II. In this case the dissociation was incomplete. Between the mass and the adjacent normal lung there was a large bullous cyst. This was aerated and communicated with peripheral bronchi of the normal lung and with the cystic spaces in the mass. The intervening bullous cyst appears to be of a previously unrecognised type resulting from embryonic traction tearing. This was one of the cases in which the abnormal artery was not confined to the mass but supplied also adjacent normally connected lung. The mid-basal sector was supplied by a branch of the normal pulmonary artery, whilst the post-basal sector was supplied by a branch of the abnormal artery. As indicated in figs. 3 and 4 both these branches showed evidence of traction. The branch of the normal pulmonary artery to the middle sector was stenosed at its origin, whilst the branches of the abnormal artery to the posterior sector met their corresponding bronchi only in their terminal twigs, as though during development their respective stems had been pulled apart. Similar evidence was present in some of the other cases. Thus in case VII the abnormal branch to the posterior basal sector converged on instead of running parallel with the corresponding bronchus. In case VI there was stenosis of a lateral branch of the normal pulmonary artery. In case III there were intervening aerated traction cysts (fig. 7).

These various findings would appear to result from long-continued traction between the sequestrated mass, anchored down by the abnormal blood supply, and the normal lung, slowly shifting from its original position in the vicinity of the septum transversum. This evidence does not prove it but it suggests that sequestration itself

is also the result of traction exerted through the capillaries of the adventitious blood supply. The bulbous shape of the bronchial tips of the embryonic bronchial tree would provide good purchase for the enveloping capillary net. Cases in which the abnormal artery supplies normal lung (with or without intralobar sequestration) are important in this respect. They give priority to the vascular component of the dual abnormality and make sequestration something in the nature of an accident, which may or may not happen when the developing lung acquires its adventitious blood supply. Moreover the mechanism by which this probably occurs (traction on embryonic blood vessels due to developmental shift) gives a clue to the genesis of various other congenital defects. It is proposed to deal with these in a subsequent paper.

SUMMARY

Seven cases of intralobar sequestration of the lung are described. In each there was an abnormal artery from the aorta supplying a bronchopulmonary mass or cyst which was dissociated from the normally connected bronchial tree. The abnormal artery was elastic and of systemic pulmonary type. There were adhesions, but the abnormality was situated in the line of the pulmonary ligament. In two cases the abnormal artery supplied adjacent sectors of the normally connected lung.

It is considered that the abnormal artery was the prior lesion and cases are described in which it was present by itself. Sequestration results from separation of a bronchial tip of the embryonic bronchial tree and is attributed to traction due to the adventitious blood supply.

Pulmonary sequestration occurs in various degrees—complete, intralobar and extralobar—dependent on the stage in embryonic life at which it occurs.

Only three cases of the intralobar type have been previously described, but the entity is now being encountered with increasing frequency since it predisposes to sepsis and requires surgical treatment. Its incidence in 280 pulmonary excisions was 5 (*i.e.* 1·7 per cent.).

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MONOCYTIC LEUKÆMOID REACTION ASSOCIATED WITH TUBERCULOSIS AND A MEDIASTINAL TERATOMA

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(Plate LXXVI)

Although monocytic leukæmia is now a well recognised clinico pathological entity, the monocytic leukæmoid reactions have received little attention in the English literature   The origin of the monocytes and the ætiology of the leukæmias are still subjects for discussion , therefore any case presenting unusual features is worthy of being recorded   •

Case report

Clinical summary

A young man aged 16 came to hospital oh account of a skin eruption of two months' duration, which was diagnosed as erythema nodosum   A skiagram of his chest revealed a curious triangular shadow protruding to the left from the middle of the mediastinum   About this time a few moderately enlarged glands were noticed in the neck and left axilla   Within a week the patient developed necrotic ulcers in the mouth, with a high temperature   A Mantoux test showed a faint reaction at 1 10 000 and was strongly positive at 1 100   After temporary improvement the mouth condition again became troublesome and after about two months the general state of the patient had greatly deteriorated , the chest signs, both clinically and radiologically, were more extensive and the patient was emaciated and very anæmic   Towards the end he developed a purpuric rash over the abdomen and iliac crests and the spleen became palpable , a large retinal hæmorrhage heralded the end and he died exactly three months after coming to hospital

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Laboratory investigations

This case was interesting hæmatologically from the beginning in that there was evidence of degeneration of the polymorphs (vacuolation) and of the red blood corpuscles (anisocytosis and poikilocytosis) at a time when the hæmoglobin was still 86 per cent   At the same time an absolute increase in both monocytes and polymorphs was found, although no attempt was made to draw conclusions from these early observations   Repeated blood counts revealed a steady decrease in hæmoglobin and red cells, with a rising leucocyte count, especially the monocytes, which reached a final figure of 36,490 per c mm   The blood counts

are recorded in table I. Two sternal punctures, performed at an interval of 8 weeks, showed a rise in monocytes from 9 to 26·6 per cent. (table II), the latter three weeks before death.

TABLE I

Hæmatological findings

Date	Hæmoglobin	Red cells (million per c.mm.)	Colour Index	White cells per c.mm.	Polymorphs per c.mm.	Lymphocytes per c.mm.	Monocytes per c.mm.	Eosinophils (per cent.)	Metamyelocytes (per cent.)	Myelocytes (per cent.)	Plasma cells (per cent.)	Blast cells with nucleoli (per cent.)	Nucleated red cells per 100 white cells.	Remarks
17.7.44	86	4·18	1·02	4700	2068	1574	916	2·0						Anisocytosis and poikilocytosis. Vacuolation of polymorphs
25.7.44	78	4·10	0·96	9000	4590	3780	360							
10.8.44	70	3·37	1·03	7000	3885	231	2555	0·5					1·0	Vacuolation of polymorphs and toxic granulation
15.8.44	56	3·62	0·77	10,900	5232	2398	3270							
23.8.44	48	2·4	1·0	10,000	5000	1800	2700	3	0	1	1			
13.9.44	40	1·9	1·05	52,000	19,240	3120	23,920		1			1	1	
22.9.44	48	2·3	1·05	48,000	15,360	3360	26,400	1·0	5				1·5	Vacuolation of polymorphs and monocytes
26.9.44	44	2·0	1·1	65,000	29,250	11,800	29,900	1·0						
6.10.44	34	1·67	1·01	82,000	38,540	4920	36,490			2		0·5	3	Vacuolation of polymorphs very pronounced

The patient had very little sputum, so this was only once examined for tubercle bacilli, with negative result. The Wassermann and Paul-Bunnell reactions were both negative. Bacteriological examination of swabs from the throat and mouth ulcers revealed nothing of importance. Biopsy of an epitrochlear gland showed chronic lymphadenitis with sinus reticulosis.

TABLE II

Cell count of bone marrow from sternal puncture

	24.7.44	19.9.44
Hæmohistiocytes	2	0·6
Megaloblasts A	0	0·2
Normoblasts A	0 } 32 per cent.	0·8 } 13·3 per cent.
,, B	8	3·7
,, C	24	8·6
Myeloblasts	2	3·4
Myelocytes, neut. . . .	6	4·3
Metamyelocytes	8 } 48 per cent.	0·0 } 46 per cent.
Polymorphs	30	36·2
Eosinophils	2	2·1
Lymphocytes	7	6·4
Plasma cells	2	1·6
Monoblasts	0	5·5
Monocytes	9	26·6
Megakaryocytes	a few	none
No. of cells counted . . .	500	1000

Necropsy report

The body was extremely pale and very emaciated. There was a purpuric eruption over the abdomen and a few petechiæ in the skin of the right forearm and on the back.

Occupying almost the whole of the left side of the chest was a tumour measuring 8×6×5 inches and weighing 3 lb. 14½ oz. It had grown outwards and downwards from the posterior mediastinum, pushing the heart and pericardium to the right so that they lay in a position antero-lateral to the tumour and almost wholly behind the sternum. At the upper limit of the pericardium, the tumour had penetrated into the pericardial sac. It had also pushed the lower lobe of the left lung before it, and all that remained of this structure was stretched like a thin piece of cloth over the upper left side of the tumour. Above this lay the small upper lobe of the lung, weighing 11½ oz. It contained a small caseous area near the hilum. The surface of the tumour was nodular and cystic. A homogeneous colloid-like substance filled the cysts, which were surrounded by pinkish grey tissue. There were no teeth, hairs or sebaceous material.

The right lung was riddled with small ill-defined nodular areas, about ¼ inch in diameter, which projected slightly from the cut surface, but otherwise differed little from the grey colour of the rest of the lung. Only a very slight excess of fluid was found in the pleural and pericardial sacs. The heart was small, weighing only 8½ oz. There were a few subepicardial petechial hæmorrhages, but no other macroscopic abnormalities were found.

The liver weighed 3 lb. 12¾ oz. Several small roughened nodules protruded from its surface and extended a short distance into its substance ; they were each surrounded by a slightly hæmorrhagic zone. No similar foci were found deep in the liver. The spleen weighed 14¼ oz. There were no macroscopic tubercles in spleen, kidneys or adrenals.

The base of the appendix was bound down to the cæcum by adhesions. Adjacent lymphatic glands were enlarged to about ¾×½×¼ inch in diameter. The muscular wall of the terminal portion of the ileum was greatly thickened and showed areas of caseation, while large, white, nodular lymphatics could be seen coursing along under the peritoneum. The mesenteric lymph glands were greatly enlarged, forming a mass 5×2×1½ inches. They felt firm, and on section showed numerous large yellow areas in their interior. There were slightly enlarged glands on both sides of the neck and in the left axilla, and a few in the mediastinum.

A small cerebral hæmorrhage was present in the left frontal lobe. The bone marrow in a portion of the shaft of the left femur appeared abnormal, being pinkish grey in colour.

Histology

The mediastinal tumour had the characters of a teratoma. Though composed largely of cystic spaces lined by actively secreting columnar epithelium, elements from all three germinal layers could be found, including neuroglial tissue, stratified squamous epithelium and angiomatous tissue. Areas of necrosis were present, and a few of the blood vessels were thrombosed. In the stroma surrounding all these elements small aggregations of monocytes similar to those seen in the spleen, bone marrow and liver were found. Monocytes were also found packed in many of the smaller blood vessels. In other parts polymorphs, lymphocytes and plasma cells were present along with the monocytes.

The tuberculous nature of the caseating area at the root of the upper lobe of the left lung was confirmed. The nodules in the right lung proved to be patches of tuberculous bronchopneumonia. The cells which filled the alveoli of the pneumonic areas were predominantly monocytes (fig. 1), with a few alveolar (phagocytic) cells and only an occasional polymorph. There was very little surrounding lymphocytic reaction, the central part of many of these areas was necrotic and a section of one of them revealed enormous numbers of tubercle bacilli.

The nodules protruding from the surface of the liver were small foci of caseating tuberculosis (fig. 2). Around these areas the sinusoids contained many cells which were chiefly monocytes; other cells present were lymphocytes, red blood corpuscles, a few polymorphs and one or two eosinophils. Elsewhere, foci of monocytes and lymphocytes could be seen, and in the centre of one of these foci there were a few endothelioid cells with pale oval nuclei and pink-staining nucleoli. In the portal tracts there were a few foci of monocytes, along with lymphocytes, polymorphs and a few plasma cells. The liver also showed chronic venous congestion with some degree of fatty degeneration. In parts the liver cells were much shrunken and had almost disappeared.

The Malpighian bodies in the spleen were small and inconspicuous, and the lymphocytes of which they were formed appeared to be largely crowded out by monocytes. The sinuses were filled with monocytes, red blood corpuscles and some polymorphonuclears. A few multinucleated cells of megakaryocyte type were also present. Several small areas of necrosis were scattered throughout the section, and in one or two places there were giant cell systems accompanied by endothelioid cells.

The thickened wall of the lower ileum showed areas of caseating tuberculosis involving all three coats. The mucous membrane was largely replaced by granulation tissue in which were many thin-walled blood vessels packed with monocytes (fig. 3) and set in a stroma containing numbers of lymphocytes, monocytes and, in places, many endothelioid cells. In addition to showing large areas

LEUKÆMOID REACTION IN TUBERCULOSIS
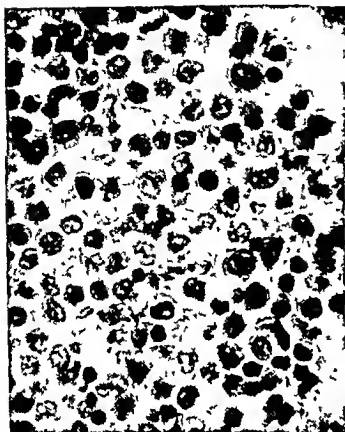
Fig 1—Lung showing alveolar oxudate, which consists largely of monocytes ×700

Fig 2—Edge of caseating tuberculous nodule in liver ×700
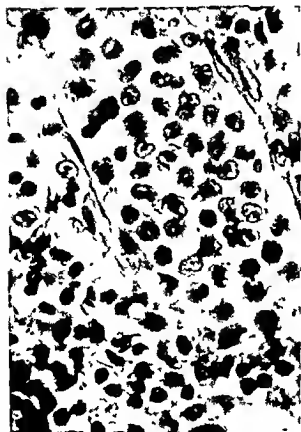
Fig 3—Ileum, showing a thin walled blood vessel packed with monocytes ×700
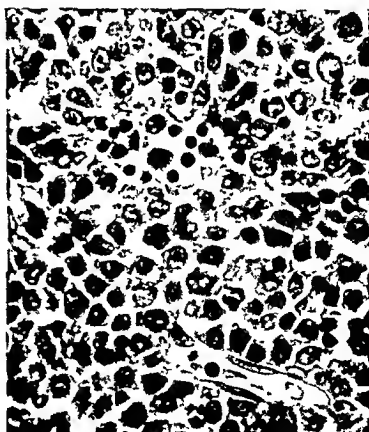
Fig 4—Bone marrow packed with monocytes ×700

of caseation, the whole of the muscular coat was diffusely invaded by monocytes, lymphocytes, plasma cells and fibroblasts. The peritoneal coat was thickened and consisted of a loose tissue with scattered cells, predominantly plasma cells and monocytes. Lymphocytes and isolated multinucleated cells were also present, but there were very few polymorphonuclears. A nodule in the peritoneal coat was caused by a thrombosed blood vessel, together with an area of necrosis surrounded by monocytes, lymphocytes, plasma cells and fibroblasts. Tubercle bacilli were found in Ziehl-Neelsen-stained sections. The appendix was normal.

The kidneys showed post-mortem autolysis and an infarct but no tubercles. There was no extravascular infiltration with monocytes but a few of the blood vessels were packed with them. The suprarenals were normal.

The bone marrow of the femur (fig. 4) was packed with cells which closely resembled the monocytes found elsewhere in relation to the tuberculous lesions. They were large polygonal cells, having a moderate amount of cytoplasm. The nucleus, which filled about two-thirds of the cell, was pale and vesicular and had a sharply defined margin but no nucleolus; in shape it was round, oval, kidney shaped or lobulated. Scattered among these cells were a few foci of cells of the red cell series and isolated polymorphs and eosinophils. Some cells showing mitotic division were present, but no megakaryocytes. One small area of necrosis was also seen.

Summary of autopsy findings

A large teratoma filled the left side of the chest. An area of fibro-caseous tuberculosis was found in the left lung and tuberculous bronchopneumonia in the right lung. Generalised tuberculous lymphadenitis was present and the mesenteric glands were particularly affected. Caseating and ulcerating tuberculosis was found in the lower ileum, and there were microscopic tubercles in the spleen. Tubercle bacilli were demonstrated in the mesenteric lymph nodes, ileum and lung. The bone marrow was packed with monocytes, and the splenic sinuses also were filled with these cells. The alveolar exudate in the lungs, the stroma of the teratoma, and many of the smaller blood vessels contained numerous monocytes. There was no real leukæmic infiltration in lungs, liver, kidneys or suprarenals; in lungs and liver monocytes were present only in relation to tuberculous foci.

DISCUSSION

The association of leukæmia, especially myelogenous leukæmia, with tuberculosis is common, Jaffé (1933) supporting the view that the debility caused by the leukæmia lights up an old tuberculous focus. Custer and Crocker (1932) and Mills and Townsend (1937),

however, report cases which they consider to be leukæmoid rather than leukæmic. It can be a matter of considerable difficulty to distinguish between these two conditions, especially if anæmia is also present, as in leuco-erythroblastic reactions. The term leukæmoid implies a blood picture resembling one or other of the leukæmias. Whereas, however, in the true leukæmias no causal agent has as yet been recognised, in cases exhibiting leukæmoid reactions a tangible exciting cause is present. This may be and frequently is an infection, or it may be an intoxication or a bone disease such as secondary carcinomatosis. As in true leukæmias, the term leukæmoid also implies the presence of immature or atypical white blood corpuscles in the peripheral blood, and there is usually a considerable increase in the total leucocyte count, though counts of over 100,000 per c.mm. are seldom seen except in chronic (true) leukæmia (Heck and Hall, 1939). In clinical hæmatology all gradations in the blood picture can be seen from a simple leucocytosis to leukæmia, these two being linked by high leucocytoses and leukæmoid reactions. Histologically the two can be distinguished by the fact that in true leukæmia there is leukæmic infiltration of most of the organs, but in leukæmoid reactions there is no such infiltration. In both conditions, however, the bone marrow may be hyperplastic.

Since such leukæmoid reactions are recognised as affecting the myeloid series of cells, it is reasonable to suppose that lymphocytic and monocytic leukæmoid reactions may also be encountered. Such reactions have been discussed by Landon (1925) and Fitz-Hugh (1931-32), and indeed it is possible that some reported as myeloid or myeloblastic (Roth, 1913 ; Marzullo and DeVeer, 1931) may rather have been of the monocytic type. That tuberculosis may produce a monocytosis of considerable degree is also indicated by experimental evidence. Cunningham *et al.* (1925) have shown that some chemical substance in the bodies of tubercle bacilli stimulates the production of the reticular cells from which, they believe, the monocytes and endothelial cells are derived, and their experience led them to think that there is a correlation between the number of monocytes in the circulating blood and the number concerned in the tuberculous process in the tissues. Since the lymph glands constitute one of the main sources of reticular cells, and assuming their theory of the origin of monocytes from them to be correct, it is hardly surprising that cases of tuberculous lymphadenitis should tend to show high monocyte counts. It has also been thought (Feldman and Stasney, 1937) that the bone marrow might become allergic in much the same way as the skin in tuberculous subjects ; some confirmation of this was obtained experimentally.

Cases of tumour formation associated with monocytic leukæmia have been reported (Haining *et al.*, 1935; Campbell *et al.*, 1936), but some at least of these are probably examples of excessively large leukæmic deposits. Ugriumow (1928), however, reported a case, regarded by him as one of monocytic leukæmia, which showed a

considerable similarity to mine in that there was a teratoma of the mediastinum : there was, however, no evidence of tuberculosis. He thought that the stroma of the tumour was closely related to the reticulo-endothelial tissue and had played its part along with the spleen, lymph glands and other parts of the reticulo-endothelial system in the production of the blood changes.

SUMMARY

1. A case is reported in which a high monocyte count (36,000 per c.mm.) was associated with wide-spread tuberculosis, particularly of the mesenteric glands, lungs, lower ileum and liver.

2. Many tissues showed extensive mononucleosis, chiefly in relation to the tuberculous lesions, and the bone marrow also was packed with these cells.

3. A large mediastinal teratoma was an interesting incidental finding.

4. It is concluded that the case was probably one of monocytic leukæmoid reaction to generalised tuberculosis and not a true monocytic leukæmia.

5. The significance of the term leukæmoid reaction is examined and the distinction of such a blood picture from true leukæmia is discussed.

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ON THE ESTIMATION OF FIFTY PER CENT. END-POINTS IN SEROLOGICAL TITRIMETRY

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IN connection with studies on the antigenic properties of the antibody globulin, I have been obliged to determine the titre curves· for agglutinins and precipitins in rabbits. As the size of the material precluded the use of exact and time-consuming methods, such, for instance, as the determination of the antibody nitrogen, there remained only the possibility of calculating the serum titres from the results of the ordinary agglutination and precipitation reactions. In doing so I arrived at a simple mathematical basis of calculation, which proved to be so eminently practicable that it is considered worthy of publication.

In Widal's serum test it is customary to grade the agglutination from 0 to 5 by ocular inspection under a lens. The agglutination of a serum in the dilutions 1 : 200, 1 : 400, 1 : 800, 1 : 1600, 1 : 3200, 1 : 6400, and 1 : 12,800 has, for instance, been expressed as 5, 5, 5, 3, 2, 1 and 0, and the strength of the serum has been regarded as represented by the titre in the last tube showing a 3, in this example 1 : 1600. It is evident, however that this method gives only a very approximate idea of the strength of the serum. By letting one single tube determine the titre, the determination may be subject to a large error, and the degree of agglutination in the surrounding tubes will not be mirrored in the result, which will be very sensitive to chance variations. Moreover, the dilution interval between the tubes is great, as the titration follows a geometrical progression. This error can naturally be reduced by repeating the agglutination with a smaller dilution factor, but then it must not be overlooked that the difference in degree of agglutination between the tubes is small, a fact which renders the determination of the end-point much more difficult. In the case of agglutination, it is not easy to express the titre by the final end-point, *i.e.* the dilution in the last tube where agglutination is perceptible.

The titre corresponding to a certain standard agglutination (of Salmonella bacteria) has been calculated by Dreyer and Inman (1917) by determining an interpolation reading from the degree of agglutination in the tube on each side of the " end-point ". The average of

the titres that could be calculated from the two tubes in this way was taken as the dilution giving standard agglutination. For the titration of therapeutic and toxic substances in animals Gaddum (1933) has pointed out the suitability of choosing as end-point the dilution at which 50 per cent. of the animals react. This end-point is less influenced by chance variations than any other, particularly the 100 per cent. end-point ordinarily used. The 50 per cent. end-point can be calculated simply according to a mathematical method described by Reed (1936) and applied to animal tests by Reed and Muench (1938). In view of the limitations of the customary methods of estimating serological end-points, this method, which is based on accumulated addition and interpolation, has been used here as a basis for the appraisement of titrations and for the determination of the strength of sera. Compared with the technique generally used, where the degree of agglutination in one or at the most two dilutions is determined, this method allows the degree of agglutination in a number of dilutions to influence the result, which thus gains reliability.

Method

The agglutinating serum is diluted two-fold in a suitable series, antigen is added and the tubes are incubated. After twenty-four hours the degree of agglutination in the various tubes is read off and graded as follows :—

100 per cent. agglutination.	Large amount of agglutinate and clear supernatant fluid.	5
80 ,, ,,	Large amount of agglutinate but not quite clear supernatant fluid.	4
60 ,, ,,	Fair amount of agglutinate consisting of large floccules and clouded supernatant fluid.	3
40 ,, ,,	Small amount of agglutinate consisting of fairly small floccules.	2
20 ,, ,,	Very small amount of agglutinate consisting of small floccules visible through a magnifying glass.	1
0 ,, ,,	No agglutinate.	0

The results in the last four tubes containing visible agglutination were noted and the titre was calculated from the combination of figures thus obtained. The case shown in table I will demonstrate the method of calculation : t is an arbitrary dilution.

The readings for the four dilutions of serum graded in units according to the standards given above will be found under + in column b. If these readings are subtracted from 5, an inverted or "negative" gradation is obtained, which will be found under — in the same column. For each dilution the sum of all positive units for that and higher dilutions is found under + in column c, and the sum of all negative units for that and lower dilutions is found in the same column under —. It is assumed that the number of units decreases continuously as the dilution increases. The 50 per cent. end-point will now lie where the total of all positive units equals the total of all negative units.

TABLE I

Method of calculation

a	b		c		d	e	f
Dilution	Reading		Accumulated addition		Percentage +	Proportionate distance between 50 per cent end-point and the percentage for the next lower dilution	Final calculation (as dilutions increase on a logarithmic scale)
	+	−	+	−			
1 : 1 t	5	0	14	0	100		log 4 = 0·6021
1 : 2 t	5	0	9	0	100		0·3187.log 2 = 0·0950
1 : 4 t	3	2	4	2	66·7	$\frac{66·7-50·0}{66·7-14·3}=0·3187$	Total (log for titre) 0·6980
1 : 8 t	1	4	1	6	14·3		Titre 4·09 t

This can be illustrated graphically as shown in the accompanying figure, where the 50 per cent. end-point is represented by the point of intersection between the curves for the values given in column c.
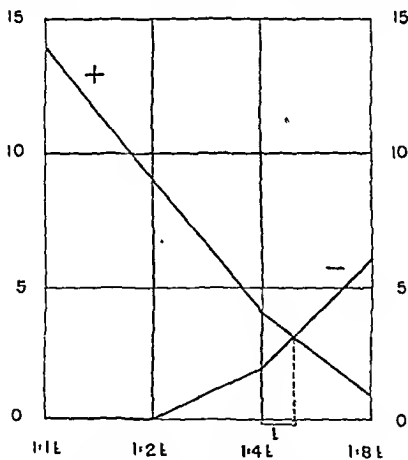
Graphic illustration of the 50 per cent. end-point.

The proportionate distance l in this figure can be calculated as shown in column e above. In the final calculation (column f) the logarithm for the final titre is obtained as the sum of the logarithm for the next lower dilution under that corresponding to the 50 per cent.

reaction, and the logarithm for the dilution factor multiplied by the proportionate distance. The hypothetical result of a titration, given in table II, illustrates the use of the figure obtained.

TABLE II

Hypothetical example of the method of calculating the result of a titration

Dilution	1 : 160	1 : 320	1 : 640	1 : 1280	1 : 2560	1 : 5120
Reading	5	5̲	5̲	3̲	1̲	0
The underlined combination of figures gives (calculated as above)	4·99 t					
Final titre (titre for the 50 per cent. end-point)	1 : 4·99 × 320 = 1 : 1597 or, rounded off, 1 : 1600					

In order to facilitate quick reading, table III shows the combinations of figures that may occur as gradations for the last four tubes with visible agglutination, and after these is given the figure by which

TABLE III

Table showing the combinations of figures which may occur as gradations for the last four tubes with visible agglutination, together with the figure by which the titre in the first tube of the four has to be multiplied in order to obtain the titre corresponding to a 50 per cent. reaction

5 5 5 5	(11·31)	5 4 2 1	3·58	4 3 3 2	4·00
5 5 5 4	10·38	5 4 1 1	3·08	4 3 3 1	3·44
5 5 5 3	8·98	5 3 3 3	5·19*	4 3 2 2	3·17
5 5 5 2	7·13	5 3 3 2	4·49	4 3 2 1	2·83
5 5 5 1	6·17	5 3 3 1	4·00	4 3 1 1	2·48
5 5 4 4	9·52	5 3 2 2	3·56	4 2 2 2	2·68*
5 5 4 3	8·00	5 3 2 1	3·18	4 2·2 1	2·33
5 5 4 2	6·42	5 3 1 1	2·75	4 2 1·1	2·00
5 5 4 1	5·66	5 2 2 2	3·08*	4 1 1 1	1·68*
5 5 3 3	6·86	5 2 2 1	2·69	3 3 3 3	(4·00)
5 5 3 2	5·66	5 2 1 1	2·30	3 3 3 2	3·44*
5 5 3 1	4·99	5 1 1 1	1·83*	3 3 3 1	2·98*
5 5 2 2	4·66	4 4 4 4	(8·00)	3 3 2 2	2·83
5 5 2 1	4·00	4 4 4 3	6·13*	3 3 2 1	2·52
5 5 1 1	3·36	4 4 4 2	5·28*	3 3 1 1	2·23
5 4 4 4	8·72*	4 4 4 1	4·76*	3 2 2 2	2·32*
5 4 4 3	6·95	4 4 3 3	5·19	3 2 2 1	2·00
5 4 4 2	5·81	4 4 3 2	4·49	3 2 1 1	1·78
5 4 4 1	5·19	4 4 3 1	4·00	3 1 1 1	1·52*
5 4 3 3	5·95	4 4 2 2	3·59	2 2 2 2	(2·00)
5 4 3 2	5·04	4 4 2 1	3·23	2 2 2 1	1·76*
5 4 3 1	4·47	4 4 1 1	2·83	2 2 1 1	1·54*
5 4 2 2	4·00	4 3 3 3	4·54	2 1 1 1	1·31*
				1 1 1 1	(1·00)

the titre in the first tube of the four is to be multiplied in order to obtain the titre corresponding to a 50 per cent. reaction. For the sake of completeness series like 2222 and others have been calculated,

but in these cases the results (which have been bracketed) should not be used, as the method is inapplicable to series too far removed from a gradually declining curve. In some of those cases, far from feeling safe in calculating an end-point, one should suspect a serological anomaly, for example that the series contained 2 agglutinins of different reactivities with the feebler reactive antibody in greater concentration. For similar reasons the results marked with an asterisk should be used with caution.

As will be shown in a subsequent paper, this method is naturally not limited to the agglutination reaction, but can also be used, for instance, in the precipitation reaction, where, however, it is of less value, as the optimal ratio of Dean and Webb (1926) provides a more correct measure of antibody content. It should be pointed out that the determination of the degree of agglutination naturally requires a certain amount of training. For this reason it is advisable at first to determine the mean value for two calculations of identical titrations.

Summary

A simple mathematical method is applied to the problem of estimating 50 per cent. end-points in serological titrimetry and a table which enables a rapid calculation to be made is suggested.

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STUDIES ON THE EFFECT OF THIOUREA AND ALLIED SUBSTANCES ON THE THYROID GLAND AND OTHER ORGANS IN RATS AND MICE

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(Plates LXXVII and LXXVIII)

The results of recent investigations have drawn attention to a group of drugs which produce hypothyroidism and goitre in rats, mice and dogs (MacKenzie and MacKenzie, 1943; Astwood, 1943). These include sulphonamides and various derivatives of thiourea. It has been suggested that they interfere with the synthesis of diiodotyrosine, so that the thyroid changes may be compensatory in nature (Campbell *et al.*, 1944). Inhibition of amphibian development by thiourea has been reported by Gordon *et al.* (1943).

In this paper an investigation of the cytological and histological changes induced by thiourea and some of its derivatives in the thyroid gland and various other endocrine organs (including the sex organs) of rats and mice is described. Some information is given about toxic effects arising from the administration of these compounds. The influence on the metamorphosis of tadpoles by feeding them with the thyroids from the treated rats was also studied.

METHODS AND MATERIALS

Albino rats from Glaxo Wistar stock were used, controls being kept in parallel. In one experiment the treated animals were litter mates : for the other experiments rats were taken at random. Both males and females were used, the body weights ranging from 90 to 330 g. Albino and champagne laboratory mice were used in experiments with repeated higher doses of thiourea and were kept under the same conditions as the rats. For the experiments with repeated high doses of thiourea and α-naphthyl thiourea over a long period, the conditions differed slightly, owing to the necessity of the writer working in a different part of England, but this had only a negligible effect on the results. The animals were fed thrice weekly on bean mash consisting of a mixture of 35 per cent. bean, 25 per cent. ground oats, 15 per cent. palm kernel meal, 10 per cent. maize mash, 5 per cent. fish meal, 0·5 per cent. meat meal, 5 per cent. dried skim milk, 2·5 per cent. dried grass meal, 1·25 per cent. dried yeast and 0·75 per cent. salt. To this 2 per cent. of cod liver oil was added. Crushed oats

and Spillers " Winalot " wholemeal biscuit were given four times a week and rat cubes having the same ingredients as the mash but with a higher proportion of dried milk once a week. Tap water was available in unlimited quantities. Five rats were kept in each cage under hygienic conditions, in semi-darkness and at a temperature of about 60° F.

The chemical compounds used were thiourea, α-naphthyl thiourea, β-naphthyl thiourea, αα-dinaphthyl thiourea and ββ-dinaphthyl thiourea. Thiourea has quite a high solubility in water and was used as 1 and 9 per cent. solutions; the other compounds were used as 0·25, 1 or 2 per cent. suspensions made with gum tragacanth. The doses were administered to rats either orally or subcutaneously but only subcutaneously to mice. If the dose were given orally the rats were first anæsthetised with ether and the compound was given by means of a rubber catheter passed into the stomach. Subcutaneously, the injection was given in the flank, near the thigh, the side being varied periodically. Mice were injected in the mid-dorsal line above the pectoral girdle. Some deaths occurred but animals surviving the period of the experiment were killed rapidly with an overdose of ether and the tissues were removed and fixed immediately. The animals were weighed at the beginning of the experiments and if repeated doses were being given they were weighed daily for adjustment of the dose. Careful autopsies were made and pleural fluid, when found, was measured. After the repeated doses of α-naphthyl thiourea the endocrine and sex glands were weighed.

A group of 3 rats was anæsthetised daily over a period of 23 days and killed by an overdose of ether, while a corresponding group was killed by a blow on the head. The glands were removed and fixed for the demonstration of any definable change in the Golgi apparatus comparable with that found by Watzka (1939).

The various glands were fixed in formol-alcohol, formol-saline or formol-Zenker. For the demonstration of the Golgi apparatus Aoyama was found to be the most satisfactory fixative (20 hrs.). Sections were stained with Ehrlich's hæmatoxylin and eosin and by Bensley's method for thyroxin. For staining the Golgi apparatus the tissue was rinsed in distilled water and then transferred to 1·5 per cent. $AgNO_3$ for a further twenty hours at 22° C. The material was again rinsed in distilled water, then reduced in Ramon-y-Cajal's solution for 5-6 hours, washed in running water, upgraded and embedded. Sections were cut at 2-4 μ and were treated with gold chloride followed by sodium thiosulphate and counterstained with neutral red.

Tadpoles hatched from spawn kept in the laboratory were transferred, at the internal gill stage, to aerated aqueous solutions of (a) 0·033 per cent. thiourea, (b) 0·001 per cent. α-naphthyl thiourea, (c) 0·005 per cent. α-naphthyl thiourea, (d) 0·001 per cent. Elityran (a Bayer product, each tablet containing thyroid approximately equivalent to 0·15 mg. total organic iodine and 0·09 mg. thyroxin) and (e) aerated distilled water. Approximately the same amount of *Elodea canadensis* was supplied as food in each vessel. Groups at more advanced stages were kept in aerated distilled water and fed with the thyroids of rats (a) treated with repeated doses of 10 mg./kg. of α-naphthyl thiourea subcutaneously over periods of 10, 12, 14, 16 and 18 days, (b) treated with repeated subcutaneous injections of 10 mg./kg. α-naphthyl thiourea over a period of 42 days, (c) given 8 oral doses of 1000 mg./kg. of thiourea over a period of 21 days and (d) kept as controls. These tadpoles were kept for 14 to 75 days. They were photographed at various stages and microphotographs were taken of sections of the thyroid and adrenal glands from the experimental rats. Drawings were made with the help of a Zeiss drawing prism, using a 1·8 U.S. Bausch and Lomb objective on a binocular with 10 × eye-pieces.

RESULTS

Toxic effects

(i) *α-Naphthyl thiourea.* Thirty-six rats were given single oral doses ranging from 4 to 35 mg./kg. The lethal dose necessary to produce 50 per cent. of deaths (L.D. 50) was found to lie between 25 and 30 mg./kg. There was no noticeable change in the behaviour of the treated rats but growth was impeded. In the controls the average weight rose from 98·4 to 187·1 g. and in the treated rats —receiving 33 doses over a period of 42 days—from 105 to 154·73 g. This is equivalent to a mean percentage increase in the treated rats of $54\pm3\cdot7$ per cent. and in the controls of $98\pm3\cdot6$ per cent. The difference of 44 per cent. is more than 8 times its standard error (5·2), and is therefore highly significant. A fatal dose of α-naphthyl thiourea led to pulmonary œdema and pleural effusion, which began within 5 hours of administration but resolved in non-fatal cases by the fourth day. The amount of pleural effusion is recorded in table I.

TABLE I

Pleural effusion following a single dose (20 mg./kg.) of α-naphthyl thiourea: volume at time of death of 15 rats

Time Interval	Volume of fluid (c c)
5 hrs. (4 killed)	2·7, 2·6, 1·6 and 1·0
1 day (5 died)	11·6, 10·9, 8·3, 7·6 and 4·6
1 „ (3 killed)	13·0, 7·8 and 5·6
2 days (2 killed)	5·2 and 4·8
4 „ (1 killed)	1·3

The fluid was clear and if left in a test tube it coagulated after some hours. Repeated doses of 10 and 15 mg./kg. were given to groups of rats (29 in all) over various periods but no deaths occurred.

(ii) *β-Naphthyl thiourea.* Fifty-six rats were given single oral doses ranging from 25 to 180 mg./kg. The L.D. 50 proved to be 160-180 mg./kg. (table II). Six out of the seven animals which died had a pleural effusion. The lungs were œdematous, with some emphysema and hæmorrhages.

(iii) *αα-Dinaphthyl thiourea.* Sixty-five rats were used, but this substance was not as toxic as either α- or β-naphthyl thiourea. Single oral doses ranged from 25 to 180 mg./kg. One animal died on the fifth day after a dose of 50 mg./kg. Autopsy revealed lungs recovering from pleurisy, with commencing fibrosis.

(iv) *ββ-Dinaphthyl thiourea* Sixty-five rats were given single oral doses ranging from 25 to 180 mg./kg. One death occurred on the eighth day following a dose of 40 mg./kg. Autopsy revealed some liver damage and œdema and congestion of the lungs.

(v) *Thiourea*. Single doses of thiourea were not given. Repeated subcutaneous doses of 500 and 1000 mg./kg. were given to 10 mice:

TABLE II

L.D. 50 of β-naphthyl thiourea given orally

Dose (mg./kg.)	No. of animals treated	Died	Percentage mortality	Survival period in fatal cases (days)
25	5	0	0	...
30	3	0	0	...
35	4	0	0	...
40	5	0	0	...
50	5	0	0	...
55	5	0	0	...
80	5	0	0	...
100	4	1	25	1
120	5	0	0	...
140	5	1	20	4
160	5	2	40	1, 1
180	5	3	60	1, 1, 3

one mouse died on the third day after the second dose of 1000 mg./kg. Repeated oral doses of 20 and 1000 mg./kg. were given to 15 rats. One rat died on the 22nd day after a dose of 1000 mg./kg.

General changes in endocrine and sex glands

These observations were confined to animals receiving thiourea and α-naphthyl thiourea, as no changes had been noted in the other groups.

Thyroid gland. The normal rat thyroid is made up of follicles which may be either round or oval. The larger follicles are usually near the surface of the gland. The cells of the follicular epithelium are generally cuboidal, but in some cases low columnar, whilst in the larger peripheral follicles they may be flattened, due perhaps to the pressure exerted by the contained colloid. The nuclei are mainly spherical or ellipsoidal and may be either basally or centrally placed. The colloid is somewhat variable in staining reaction. In some cases it fills the follicular cavity completely, in others it is vacuolated throughout and yet again only at the margins. Inter-follicular epithelium is present and connective tissue forms a delicate fibrous stroma, relatively small in amount, but containing a rich capillary plexus of blood vessels. The variability in histological structure is doubtless due, in part at least, to functional changes as yet imperfectly understood (fig. 1). In the normal mouse the peripheral and central vesicles are less sharply differentiated than in the normal rat.

α-Naphthyl thiourea. (i) Single oral doses of 20 mg./kg. were given to a group of 15 rats. Five of these died on the second day and the remainder were killed at intervals of 5 hours, 1, 2 and 4 days. The maximum changes in the thyroid occurred on the fourth day, when

the epithelial cells of the peripheral vesicles ranged from cuboidal to columnar and most of the cells of the other vesicles were columnar or high cuboidal, the nuclei tending to be basal, while the colloid was vacuolated in all vesicles.

(ii) Thirty-three subcutaneous doses of 10 mg./kg. were given to 19 rats over a period of 42 days. After this dosage there was no enlargement of the thyroid. The epithelium of the peripheral vesicles was more columnar than in corresponding regions of the litter-mate controls, but both groups showed depletion of colloid with vacuolation (fig. 2). There was possibly a slight atrophy of the thyroids of the treated rats, for the average weight was 0·011 g., while that of the controls was 0·017 g.

(iii) Eight oral doses of 10 mg./kg. were given to 5 rats over a period of 9 days. Two died on the second day and the remainder were killed at the end of the period. The thyroids were not enlarged and there was little structural change. The cuboidal epithelium of the peripheral vesicles was low but in the other vesicles it was higher than normal. The nuclei tended to be more basal and the colloid rather more vacuolated than normal.

(iv) Twenty oral doses of 15 mg./kg. were given to 5 rats over a period of 23 days. The only discernible difference was in the presence of desquamated cells in the centre of some vesicles and a slight reduction in colloid.

Thiourea. (i) Eleven doses of 20 mg./kg. were given orally over a period of 14 days to 5 rats. The epithelium of the central vesicles and of many of the peripheral vesicles was columnar. There was little colloid in the central vesicles.

(ii) Five rats were given oral doses of 1000 mg./kg., two of them receiving 7 doses, the others 8 doses over a period of 20 days. The vesicles in all cases were oval rather than circular and the epithelium was columnar.

(iii) Five rats were given 20 repeated oral doses of 1000 mg./kg. over a period of 23 days. Marked hypertrophy of the glands resulted, with elongation of the lumen of the vesicles. The epithelial cells were high columnar and there was notable absence of colloid (fig. 3).

(iv) Ten doses of 500 mg./kg. were given subcutaneously to 5 mice over a period of 10 days. The epithelial cells were columnar and depletion of colloid was slight.

(v) Ten doses of 1000 mg./kg. were given subcutaneously to 5 mice over a period of 11 days. One died after the third dose on the third day; the remainder were killed on the 11th day. The thyroids did not all reveal the same amount of change, the height of the cells varying from cuboidal to high columnar, with marked depletion of colloid in one only.

Thymus. The thymuses of the rats treated with α-naphthyl thiourea showed a marked decrease in size and weight after 9, 12, 13, 15, 17 and 33 doses of 10 mg./kg. and after 20 doses of 15 mg./kg.

The mean weight of 19 thymuses from the rats receiving 33 doses was 0·12 g., from the corresponding controls 0·32 g., a statistically significant difference.

Pituitary. No change of any significance was observed in any pituitaries of animals killed 5 hours, 2 days or 3 days after a single dose of 20 mg./kg. of α-naphthyl thiourea nor after 9, 12, 13, 15 or 17 doses of 10 mg./kg.

Adrenals. The adrenal glands of the animals treated with repeated doses of α-naphthyl thiourea and thiourea showed no significant histological changes. There was a slight but not significant difference between the mean weights of the adrenals of the animals receiving the 33 repeated doses of 10 mg./kg. and the corresponding controls. The former were 0·515, the latter 0·68 g. The Golgi apparatus was clearly seen in the cells of both medulla and cortex, generally as a juxta-nuclear cap. No difference was observed between controls and treated rats.

Sex glands. There was no apparent abnormality in the testes of the rats treated with single or repeated doses of α-naphthyl thiourea or with the repeated doses of thiourea. However, the mean weight of 19 pairs of testes from the animals receiving 33 doses of α-naphthyl thiourea was 1·156 g., while that from the corresponding controls was 1·225 g. This difference does not quite reach the conventional level of statistical significance.

Cytological changes in the thyroid gland

(i) *Cell size and shape.* In the thyroids of normal rats there is variation in the shape and size of the cells. When the peripheral vesicles are full of colloid the cells tend to be low cuboidal, otherwise they are all cuboidal. The central vesicles are made up of high cuboidal or low columnar cells. No striking changes are apparent after treatment with α-naphthyl thiourea but after thiourea the cells are high columnar and the cytoplasm is not vacuolated.

(ii) *Nuclear position.* The nucleus is normally basal or central in position and no change is discernible after treatment with α-naphthyl thiourea and the lower doses of thiourea. Repeated administration of higher doses of thiourea is associated with round to oval nuclei, usually in a basal position.

(iii) *Golgi apparatus.* Over 46 thyroids were impregnated to show the Golgi apparatus. It appears that there are three main types, differing somewhat from those described by Gillman (1934): (a) a loose network which may ramify in the cell to a lesser or greater extent (figs. 4-6); (b) a condensed network (fig. 4); (c) a network apparently fragmented and in intimate connection with several small or one large vacuole which distends the cell and forces the nucleus towards the basal position (figs. 4, 5, 7 and 8). In the vast majority of cases the Golgi apparatus is situated on the luminal side of the
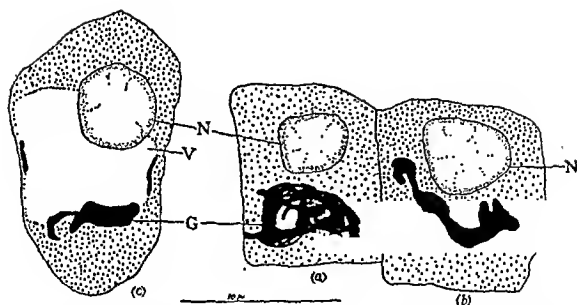
FIG. 4.—Cells from thyroid gland of normal rat showing Golgi apparatus : (c) " fragmented," (a) network, (b) condensed network. N = nucleus, G = Golgi apparatus, V = vacuole.
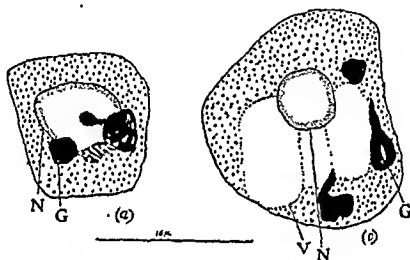
FIG. 5.—Cells from thyroid gland of rat given 20 oral doses of α-naphthyl thiourea (20 mg./kg.). N = nucleus, G = Golgi apparatus, V = vacuole.
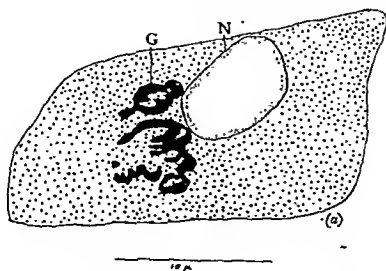
FIG. 6.—Cell from thyroid gland of rat given 20 oral doses of thiourea (1000 mg./kg.). N = nucleus, G = Golgi apparatus.

nucleus, but in a few cases there is an apparent reversal to the basal position (Okkels, 1931). According to Gillman "reversal of position" is only to be seen in single sections. Serial sections demonstrate that a "reversal" Golgi apparatus is only part of the whole, which extends around the nucleus. Ponse (1938), however, ignores this interpretation and feels forced to admit that there is a relation between the inversion of the Golgi apparatus and the basal excretion of colloid. The appearance of a vacuole within the Golgi network, the increase in size of this vacuole or the formation of several vacuoles in connection with the fragmented Golgi apparatus, the protrusion of the vacuole into the lumen of the vesicle and the presence of a vacuole outside the cell in normal and α-naphthyl thiourea-treated animals all suggest that the Golgi apparatus is connected with a secretory cycle in the cells and is concerned with the elaboration of the vacuoles. The types of Golgi apparatus observed in this sequence may represent Gillman's (1934) anabolic and katabolic types. In normal rats (fig. 7) all three types of Golgi apparatus were observed, but on the whole the loose network type (a) predominated. There was no definable difference between the Golgi apparatus in thyroids from rats anæsthetised daily over a period of 23 days and then killed by an overdose of ether and in those from rats killed by a blow on the head comparable with the difference found by Watzka (1939) in spinal ganglion cells after death from an overdose of chloroform.

There was no essential deviation from the normal in rats given single or repeated doses of α-naphthyl thiourea: all three types of Golgi apparatus could be seen (fig. 8). However, all thyroids from rats treated with thiourea showed a noticeable absence of cells with large vacuoles and fragmented Golgi apparatus. The group receiving 20 doses of 1000 mg./kg. showed no vacuolated cells, the majority having a hypertrophied Golgi apparatus in the form of a loose network, in marked contrast to the controls and those receiving α-naphthyl thiourea (fig. 9).

Tadpole experiments

In these experiments all the tadpoles in the 0·001 per cent. α-naphthyl thiourea solution were dead by the 22nd day and those in 0·005 per cent. solution by the 20th day, due, possibly, to the drug being toxic in these concentrations. During the experiment there was a distinct loss in pigmentation in comparison with the controls. The tadpoles kept in 0·001 per cent. Elityran solution had by this time begun to metamorphose and had hind limbs and front limb buds, showing an essential change as compared with the controls. The tadpoles were coming to the surface to breathe periodically, but eventually all died. Those in the 0·033 per cent. thiourea solution were smaller than the controls, but unlike them had hind limb buds. They were kept for a further period of 32 days, by which time some had died:

EXPERIMENTS WITH THIOUREA

Fig 1

Fig 7

Fig 2

Fig 8

Fig 3

Fig 9
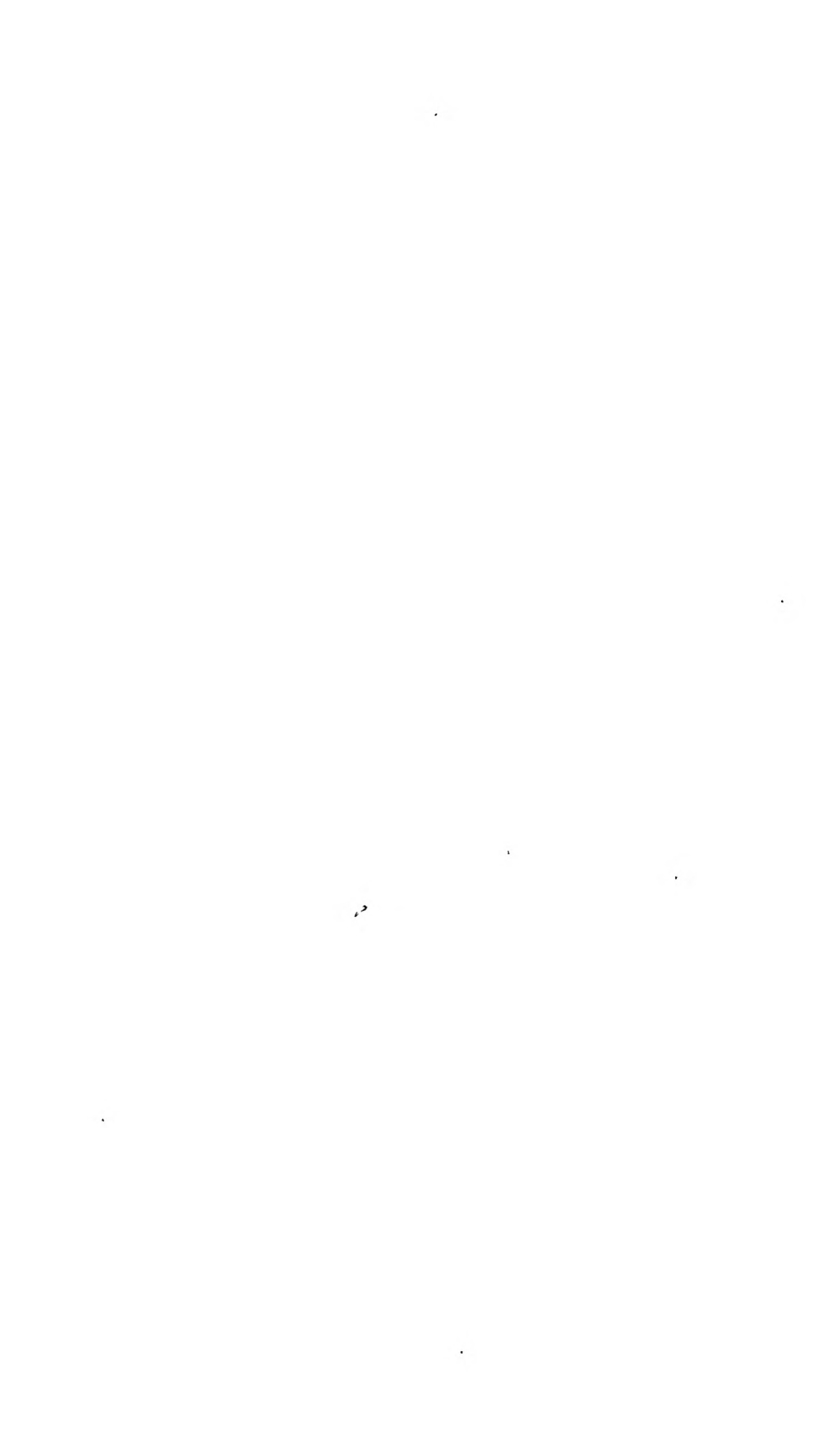
others were larger than the controls and their development was little in advance. Some showed tail abnormalities such as twisting. The results of the experiments in which groups of tadpoles were fed with thyroids of treated and control rats are summarised in tables III-V (figs. 10-15).

TABLE III

Changes in tadpoles fed with thyroids from rats given daily subcutaneous doses of 10 mg./kg. α-naphthyl thiourea and killed at intervals of 10, 12, 14, 16 and 18 days

Period of thyroid feeding (days)	15 fed with thyroids from normal rats	15 fed with thyroids from treated rats	No thyroids given (15)
14	Hind limb buds appeared	Hind limb buds appeared	No observable change
18	Increase in size	Increase in size	No change
38	Hind limbs pigmented	Hind limbs pigmented	No change

TABLE IV

Changes in tadpoles fed with thyroids of rats given 33 subcutaneous doses of 10 mg./kg. a naphthyl thiourea over a period of 42 days

Period of thyroid feeding (days)	30 fed with thyroids from normal rats	30 fed with thyroids from treated rats	No thyroids given (30)
5	Hind limb buds longer than in controls	Hind limb buds longer than in controls	Hind limb buds minute
9	Hind limb buds external · wedge shaped bodies	Hind limb buds external · wedge shaped bodies	Hind limb buds internal
18	Out of 30, 11 alive Fig 10	Out of 30, 23 alive Fig 11	Fig 12
24	1 alive	15 alive	All alive

TABLE V

Changes in tadpoles fed with thyroids of rats given 8 oral doses of 1000 mg./kg. thiourea over a period of 20 days

Period of thyroid feeding (days)	12 fed with thyroids from normal rats	12 fed with thyroids from treated rats	No thyroids given (12)
1	Limb buds evident in a few · rather wedge shaped	Limb buds evident in a few : rather wedge shaped	Limb buds in a few : no alteration in shape
9	5 alive · hind limbs external : varying in size and development	3 alive : hind limbs external and angled	9 alive · hind limb buds not angled
15	Fig 13 All dead	Fig 14 All dead	Fig 15 9 alive

DISCUSSION

It is evident from these experiments that neither $\alpha\alpha$-dinaphthyl thiourea, $\beta\beta$-dinaphthyl thiourea nor thiourea was lethal in the doses given over the various periods. The rats tolerated higher doses of thiourea than those used by Landgrebe and Morgan (1946), but, as they state, " different workers have found the toxicity of thiourea to rats to be very variable ". The allied compound, α-naphthyl thiourea, when given in a single low dose caused 100 per cent. mortality in 24-72 hours. The most striking pathological change occurred in rats after the administration of single doses of α-naphthyl thiourea—a change comparable with that noted by Richter and Clisby (1942) after administration of phenyl thiocarbamide. This change consisted of an early pleural effusion followed by pulmonary œdema and was due to a primary rather than a secondary action of the drug. The pleural effusion appeared within 5 hours and in non-fatal cases was being resolved by the fourth day. Further, the treatment with α-naphthyl thiourea produced marked atrophy of the thymus and markedly impeded growth. Repeated doses of α-naphthyl thiourea and in particular of thiourea caused an increase in the height of the cells of the thyroid vesicles. Hyperplasia of the thyroid occurred after thiourea treatment, agreeing with the findings of Astwood (1943), MacKenzie and MacKenzie (1943), Mixner *et al.* (1944) and others. Further, in animals given thiourea the nucleus assumed a basal position in the cell, possibly due to the internal vacuolar pressure resulting from the marked activity of the gland. Marked activity of the thyroid has been linked with considerable enlargement of the Golgi apparatus in hyperthyroidism and exophthalmic goitre (Ludford and Cramer, 1928-29 ; Okkels, 1931, 1932, 1934 ; Gillman, 1935-36 ; Welch and Broders, 1940). The enlargement of the Golgi apparatus found after repeated higher doses of thiourea, the increased height of the cells, general thyroid hyperplasia and depletion of colloid all seem to indicate marked activity of the thyroid glands of the rats used in these experiments. It is possible that depletion of colloid is a result of interference by thiourea with the synthesis of thyroid hormone, a suggestion put forward by Campbell *et al.*, Astwood and others.

The results of the tadpole experiments agree with those of Gordon *et al.* in so far as there was increase in size of those kept in thiourea solution, and there were abnormalities of the tail. The other tadpole experiments did not furnish enough critical evidence to allow conclusive deductions to be made as to the amount of thyroxine in the glands.

SUMMARY

1. The L.D. 50. of α-naphthyl thiourea was found to be 25-30 mg./kg., of β-naphthyl thiourea 160-180 mg./kg., of $\alpha\alpha$-dinaphthyl-thiourea and $\beta\beta$-dinaphthyl thiourea >180 mg./kg. and of thiourea >1000 mg./kg. given orally.

Plate LXXVIII

Fig. 10.—Control tadpole.

Fig. 11.—Tadpole fed with thyroids from normal rats (18th day).

Fig. 12.—Tadpole fed with thyroids from rats given 33 subcutaneous doses of 10 mg./kg. α-naphthyl thiourea over a period of 42 days (18th day).

Fig. 13.—Control tadpole.

Fig. 14.—Tadpole fed with thyroids from normal rats (9th day).

Fig. 15.—Tadpole fed with thyroids from rats given 8 oral doses of 1000 mg./kg. thiourea over a period of 21 days (9th day).

All × 4.

EXPERIMENTS WITH THIOUREA

Fig 10

Fig 11

Fig 12

Fig 13

Fig 14

Fig 15

2. Single doses of α-naphthyl thiourea produced marked pulmonary œdema. Repeated doses of 10 and 15 mg./kg. impeded growth and caused atrophy of the thymus.

3. Repeated doses of 1000 mg./kg. of thiourea caused congestion, depletion of colloid and hypertrophy of the thyroid gland.

4. Three main types of Golgi apparatus were found in normal thyroid gland cells: (a) a loose network, (b) a condensed network, (c) a fragmented network in connection with several vacuoles or one large vacuole. These may be connected with phases in the secretory cycle of the cell.

5. In the thyroid cells of rats receiving large doses of thiourea over a long period, types (a) and (b) were alone found and there was hypertrophy of the Golgi apparatus. In those from rats receiving repeated doses of α-naphthyl thiourea over a long period (a), (b) and (c) were all found.

6. Tadpole experiments point to the fact that when thiourea and α-naphthyl thiourea are administered to rats over a certain limited period there is still enough active principle in the thyroid to accelerate metamorphosis. When tadpoles are kept in thiourea and α-naphthyl thiourea solutions growth changes are exhibited in the one case and colour changes in the other.

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THE EFFECT OF PENICILLIN ON THE TUBERCLE BACILLUS

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(PLATE LXXIX)

UNTIL now the tubercle bacillus has been listed among the organisms insensitive to penicillin. The Oxford workers were the first to state this conclusion (Abraham *et al.*, 1941), which was based on failure to inhibit the organism in heavily inoculated glycerol broth. The penicillin in the test cultures was renewed every second day and the highest concentration was 40 units per c.c., and the drug was neither so pure nor so accurately standardised as it is to-day (Gardner, personal communication). In 1943 Robinson reported that mice infected with avian tubercle were not protected by penicillin treatment. Smith and Emmart (1944) carried out a more extensive investigation. They used concentrations of up to 30 units per c.c., glycerol broth and Proskauer and Back's medium, and a virulent laboratory strain of human tubercle bacillus. "No marked inhibitory effect was noted". With a chick embryo technique the number and size of the tubercles formed were reduced in the highest concentrations, but the embryos were damaged by the drug. In some animal experiments the authors say that the dose used was too small and that the drug was toxic. They conclude that "the possibility is left open that more intensive treatment might be better". While the present work was still in progress Woodruff and Foster (1945) reported that they found no inhibition with a number of mycobacteria, including an avirulent strain of human tubercle bacillus, even with a concentration of 1000 units per c.c. More recently Friedmann (1945) has recorded good growth of human, bovine and avian tubercle bacilli in the presence of penicillin of an initial strength of 20 units per c.c. The medium was Tyrode's solution containing chick embryo tissue.

In this paper I shall describe and discuss experiments which shew that under appropriate circumstances penicillin inhibits the human tubercle bacillus.

Experimental details

Pryce's (1941) slide culture method was chosen because I desired a small inoculum and wished to study the organism directly from the patient without the intervention of even a single subculture. For use it was modified on the basis of Muller's (1944) recommendations.

Under sterile conditions small screw-capped bottles of 6 c.c. capacity were filled with 3 c.c. of liquid medium. Strongly positive sputum was emulsified with sterile saline on a mechanical shaker, with glass beads to assist mixing. A standard amount (1-2 c.mm.) of the emulsion was spread on one-half of 2.5×0.5 cm. glass slides, which were then dried for two hours at 37° C., treated with 10 per cent. sulphuric acid for 15 minutes and washed in five changes of sterile distilled water. One slide was then transferred aseptically to each bottle. The cultures were incubated at 37° C. and at two-day intervals a control culture was removed. The slide was dried over a small flame and stained with strong carbol-fuchsin as in the Ziehl-Neelsen method, but without counterstaining. When good growth could be recognised under the low power (fig. 2), the test cultures were removed, fixed and stained. The finished slides can be mounted in order on a microscope slide under a coverslip. Before insertion of the slides, the required amounts of penicillin in solution were added to the test cultures. As a rule cultures grew in from 7 to 9 days. Penicillin was not added to the test cultures during this period, and was present at the end of the experiments.

Culture medium. A watery extract of Loewenstein's medium was made by adding 12 c.c. of sterile distilled water to a slope of medium as supplied by the L.C.C. Pathological Service. The slope was left for one month at room temperature and the clear fluid used as medium. This medium does not destroy penicillin, a concentration of five units per c.c. loses on an average 25 per cent. in strength in seven days at 37° C.

Penicillin. Two American preparations (Merck's and Pfizer's), and some English (T.R.C.) were used. All solutions were made up immediately before the experiment.

Muller's method of recording results was not adopted; complete absence of visible growth (fig. 3) was taken as inhibition, partial growth being disregarded. With the technique described and all glassware cleaned as recommended by Drea (1942), only 0.3 per cent. of the experiments showed contamination.

Results

In the first experiment the concentration of penicillin ranged from 1400 to 15 units per c.c. Complete inhibition was noted at strengths down to and including 70 units per c.c. In further experiments the end-point was variable with different strains and different batches of media. In table I the values refer to the concentration at the beginning of the experiment. The penicillin level was falling throughout and the minimum inhibitory concentration was not determined. It probably varies with the strain of organism and the medium, but under the conditions described it was regularly noted that inhibition takes place with initial concentrations of from 20 to 80 units per c.c.

At the end of one experiment the inhibited test cultures were re-incubated in fresh penicillin-free medium and growth appeared after eight days; in this case at least the inhibitory effect was apparently bacteriostatic.

The above experiments show two main differences from those of the other workers quoted. (1) The inoculum was very small: approximately 10,000 organisms were used per culture, of which some hundreds grew. (2) The organism was always freshly isolated from the host.

PENICILLIN AND *M. TUBERCULOSIS*
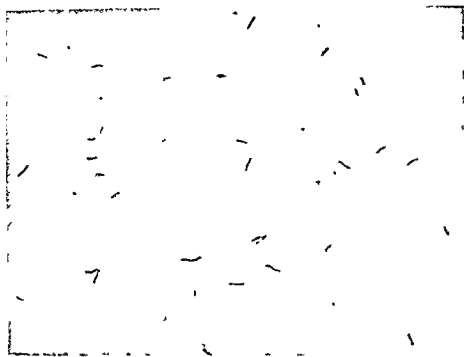
FIG. 1.—Control culture at the beginning of the experiment, showing numerous acid - fast bacilli. ×1000

FIG. 2.—Control culture after eight days' incubation, showing moderate growth. ×400
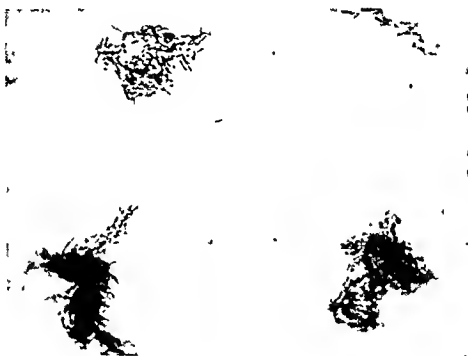
FIG. 3.—Test culture containing an initial dose of 40 units of penicillin per c c. After eight days' incubation no growth can be seen. ×1000.

Either or both of these factors could account for the difference in my results but the first is the more likely, because, according to Woodruff and Foster, the tubercle bacillus is an active producer of penicillinase; a seven-day culture of this rapidly growing avirulent strain (R 607, U.S. Nat. Col.) could destroy 800 units of penicillin per c.c. in 2 hours at 37° C. The addition of a large inoculum of such an organism to a penicillin-containing medium is equivalent to adding a quantity of active penicillinase. To test this view experi-

TABLE I

Concentration of penicillin inhibiting M. tuberculosis hominis

Strain	Penicillin (range of concentration in units)	Inhibition (lowest strength giving complete inhibition)
S	1330-15	70
B	1660-80	80
P	340-40	80
P	100-10	34
P	40	40
K	320-20	20
K	480-24	48
K	270-17	34
Q	420-10	40
O	80-2	40
O	160-10	20

ments were done with different sizes of inoculum and different organisms. Two strains were used, a recently isolated virulent strain (O), and R 607 (U.S. Nat. Col.) as used by Woodruff and Foster (vide supra). Both were grown in the watery egg medium and were incubated at 37° C. for varying periods. The inocula were measured amounts of a smooth suspension of the organism made by grinding up a weighed quantity with sterile saline. The experiment was done in duplicate.

These experiments showed that this recently isolated virulent strain could be inhibited by penicillin if the inocula were small enough (table II). If the inoculum were too large or the dose of

TABLE II

Penicillinase-like effect of large inocula of a recently isolated strain of M. tuberculosis hominis (virulent strain O)

Inoculum (mg.)	Penicillin (units per c.c.)	Growth	Penicillin at end of expt (time in days in brackets)
1	50	+++	None (7)
0 01	2	+++	None (23)
0 01	10	++	None (23)
0 01	50	—	6 units per c.c. (23)

penicillin too small the penicillin was destroyed and growth occurred. An inoculum of 0·01 mg. in 5 c.c. of medium was inhibited by 50 units

per c.c. over a period of four weeks. The next experiment (table III) demonstrated that the R 607 strain grew so rapidly and destroyed

TABLE III

Penicillinase-like effect of small inocula of a laboratory strain of
M. tuberculosis hominis *(avirulent strain R 607)*

Inoculum (mg.)	Penicillin (units per c.c.)	Growth	Penicillin at end of expt. (time in days in brackets)
0·1	50	+++	None (2)
0·001	50	+++	None (4)
0·0001	50	++	None (4)
0·00001	50	++	None (4)
0·00001	500	++	None (5)

penicillin so effectively that even 0·00001 mg. would grow in the presence of 500 units per c.c. Under the conditions of test it is therefore an insensitive organism.

Discussion

In its natural habitat the tubercle bacillus is an obligate parasite which on isolation from its host requires a complex medium. The nutritional needs of the organism are not exactly known, but it is possible by repeated subculture to make a particular strain grow first on less rich material and later on a simple medium of known composition such as Long's medium. These laboratory strains have been much used for experimental work because of their rapid growth and because standardised media could be used, but the relation between their properties and those of the naturally occurring forms is not known. Although conclusions drawn from such experiments are usually assumed by implication to apply also to the " wild " form, this may be quite untrue—a limitation of in-vitro work on the tubercle bacillus admitted by Wells and Long (1932). Because the nutritional needs are not identical in all laboratory strains their reaction to chemotherapy may also differ from that of parasitic strains. In two recent papers Youmans (1944) endeavours to show how the same chemicals may act differently on two different strains and stresses the need for an accurate in-vitro method of testing the tubercle bacillus against possible growth inhibitors—a view in accord with the evidence in this paper that experimental tests on the tubercle bacillus may be difficult to evaluate.

All the experiments on penicillin and tubercle bacilli done by the other workers quoted have the following characteristics: (1) the inoculum is large (0·1-1·0 mg., or a " loopful ") ; (2) the organisms are laboratory strains ; (3) no attempt is made either to assess the penicillin content of the cultures during or at the end of the experiment or to renew the penicillin during the course of the experiment. (The

Oxford workers are excepted. They used an old laboratory strain and a heavy inoculum, and they renewed the penicillin every two days in the test cultures, which contained 0·4, 4·0 or 40·0 units per c.c. My observations with R 607 show that even this procedure might not maintain an inhibitory level in the cultures.) The effect of penicillin on recently isolated strains is probably determined by the size of the inoculum, but an old laboratory strain like R 607 is apparently too active a penicillin destroyer to be sensitive even with so small an inoculum as 0·00001 mg., whereas a recently isolated strain is inhibited if 0·01 mg. is tested against 50 units per c.c. It has been reported for other organisms that the size of the inoculum can effect the action of penicillin (Herrell, 1945). It appears that in becoming adapted to simpler nutrients the organism has also become more resistant to penicillin, possible because it produces a penicillinase-like substance. With other bacteria it is recognised that sensitivity to penicillin does not bear a direct relation to penicillinase formation. This is probably the same for the tubercle bacillus, because the sensitive strain used in the present investigation can destroy penicillin if the inoculum is big enough (1·0 mg. against 50 units per c.c.). The ability to destroy penicillin may not be the only reason for the insensitivity of R 607.

Conclusion

With penicillin (as with sulphonamides according to Youmans) it is essential when testing the tubercle bacillus to use recently isolated virulent strains and measured small inocula. These criteria would make in-vitro experiments more difficult, but would give a more reliable estimate of the capabilities of the drug. The slide culture method is a suitable one and has the advantage of giving quick results. It has the drawback of being more elaborate and requiring strict chemical cleanliness as well as asepsis for success. Also it cannot as yet be used with media of known composition, and complex media may contain substances which react with the compound being tested. This is not necessarily a disadvantage, however, because any antiseptic must eventually be used in the tissues, where neutralising factors also exist. For this reason laked blood is perhaps the best medium (as used by both Pryce and Muller), but I have found a watery extract of an egg medium to be more reliable.

Summary

1. Recently isolated strains of *M. tuberculosis hominis* were found to be inhibited by penicillin at strengths of 20-80 units per c.c.; the effect appeared to be bacteriostatic.

2. An avirulent laboratory strain was not inhibited; indeed it destroyed the penicillin.

3. The difference between these results and those of other workers appears to be due to differences in technique and the strains used.

4. The requirements of in-vitro methods for testing *M. tuberculosis* are briefly discussed.

I should like to thank Sir Alexander Fleming, Dr D. M. Pryce and the staff of my laboratory at Harefield Hospital for their help.

Postscript

The results recorded by Ungar and Muggleton (1946), which I saw before publication, stress again the need for a careful study of the factors affecting the in-vitro growth of *M. tuberculosis*. Their work was done with a different technique and so is not directly comparable with mine. They used a laboratory strain growing on a synthetic medium with a large inoculum, and in the second set of experiments chemically pure penicillin in a weaker concentration. Their results also disagree with those of the other workers quoted. The subject requires further elucidation.

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THE EFFECT OF PENICILLIN ON THE GROWTH OF HUMAN TYPE *M. TUBERCULOSIS*

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ABOUT three years ago we observed that the addition of small amounts of penicillin to growing cultures of *M. tuberculosis* appeared to increase the rate and extent of growth. At that time it could not be determined if this was an effect of the penicillin or of unknown factors present in the penicillin as impurities. When pure penicillin became available it was decided to reinvestigate the matter more fully.

In a preliminary experiment two culture media were used, liver extract broth and a modification of Long's synthetic medium, the composition of the media being as follows :—

Liver extract broth

Potato extract	.	500 ml.
Liver extract	.	25 „

Mix, steam for 30 minutes, filter and add :—

Hartley's broth	.	500 ml.
Glycerol	.	25 „

Adjust to *p*H 7 2, bottle and sterilise by autoclaving.

Liver extract

Fat-free ox liver . . .	.	454 g.
Tap water	.	1000 ml.

Mix, render just acid to litmus, steam for two hours, filter through paper and sterilise by intermittent steaming.

Potato extract

Potatoes (minced) . .	.	4·5 kg.
Tap water	.	10 0 l.

Clean and mince the potatoes, place in a muslin bag and suspend in water overnight at room temperature, remove the bag and decant the supernatant fluid, filter and bottle , sterilise by intermittent steaming.

Synthetic medium

Glycerol	50 0 g.
Sodium citrate	6·0 „
Asparagine	5·0 „
Glycine	4 0 „
KH$_2$PO$_4$	2 0 „
Ammonium chloride	1 0 „
MgSO$_4$.7H$_2$O	0·50 „
Ferrous ammonium citrate . . .	0 05 „
Guanidine HCl (2·2 per cent. aq. sol.) . .	5·00 ml.
Distilled water to	1000 „

The containers were screw-capped 4-oz. bottles ("medical flats"), each of which contained 50 ml. of medium. The penicillin used was a T.R.C. preparation having a potency of 248 units per mg.

Penicillin dissolved in distilled water was added at two levels, 1·0 and 5·0 units per ml. of medium, and for each level six bottles were used. We purposely chose these two levels of penicillin, which are the optimal likely to be achieved in the blood of patients during penicillin treatment. Three bottles were left as controls without penicillin.

All bottles were inoculated with a virulent human type (strain 418) of *M. tuberculosis* by floating on the surface of the medium a small piece (about 2 mm. diameter) of primary culture 28 days old from liquid medium and were then incubated at 37° C. At intervals a small sample of medium was withdrawn, and, after assay, further penicillin was added when necessary to bring it up to the original concentration.

After three weeks the cultures were examined and the amount of growth recorded (table I). Cultures showing a heavy folded felt completely covering the

TABLE I

Effect of 5 units and 1 unit of penicillin per ml. on growth

	Penicillin added	Culture no.						Mean index
		1	2	3	4	5	6	
Liver broth . . .	5·0 u./ml.	4·0	4·0	4·0	4·0	3·0	4·0	3·83
	1·0 u./ml.	3·0	3·0	4·0	4·0	S	4·0	3·60
	Nil	2·0	1·5	S	...	...	...	1·75
Synthetic medium . .	5·0 u./ml.	4·0	4·0	3·0	4·0	3·0	3·5	3·58
	1·0 u./ml.	2·5	4·0	4·0	4·0	4·0	3·0	3·58
	Nil	3·0	1·5	2·0	...	...	...	2·17

S = culture sank; not included in mean.

surface of the medium were given the index 4·0; those with felt covering the surface completely but not so heavily 3·0; those with the surface just covered with an even pellicle 2·0; those with spreading growth but incomplete covering of the medium surface 1·0.

The results having confirmed our previous observation, we decided to carry out a more comprehensive experiment and also to determine whether the effect should be ascribed to penicillin or to impurities.

For this purpose two samples of Na penicillin were used: a stock penicillin having a potency of 590 u./mg. and pure penicillin having a potency of 1650 u./mg. These samples were added to bottles of the synthetic medium of the same composition as before, five bottles being used for each level.

Group 1. Control medium; no addition of penicillin.
Group 2. Penicillin 590 units/mg.; 5·0 units per ml. of medium.
Group 3. Penicillin 590 units/mg.; 1·0 unit per ml. of medium.
Group 4. Penicillin 1650 units/mg.; 5·0 units per ml. of medium.
Group 5. Penicillin 1650 units/mg.; 1·0 unit per ml. of medium.

Group 6. Penicillin 590 units/mg.; (equivalent to 5·0 units per ml.)
inactivated with *B. subtilis* penicillinase.

Group 7. Penicillinase control (same concentration of enzyme as in
group 6).

These containers were inoculated with strain 418 and incubated
at 37° C. At 48-hour intervals the medium was sampled, assayed for
penicillin and brought up to the original potency as necessary.

Three weeks after inoculation the results were recorded in a fashion
similar to that adopted previously and photographic records were
made. The cultures were then dispersed in the medium by shaking,
filtered off, " dried " by suction on a Buchner funnel for ten minutes
and weighed. The results are shown in table II.

TABLE II

*Weight and macroscopic growth of the cultures
under influence of penicillin*

Group	Penicillin added	Culture no.					Mean index	Weight of growth (g.)
		1	2	3	4	5		
1	Nil	2·0	1·0	1·5	1·0	2·0	1·5 (1·00)	2·3 (1·00)
2	590 u./mg. {5·0 u./ml.	3·0	3·0	5·0	5·0	4·0	4·0 (2·67)	6·6 (2·87)
3	{1·0 u./ml.	5·0	5·0	5·0	3·0	5·0	4·6 (3·07)	10·1 (4·39)
4	1650 u./mg. {5·0 u./ml.	4·0	5·0	5·0	5·0	5·0	4·8 (3·20)	9·0 (4·30)
5	{1·0 u./ml.	5·0	5·0	5·0	5·0	5·0	5·0 (3·33)	10·0 (4·35)
6	As 2 but inactivated	2·0	2·0	1·0	0·5	1·0	1·3 (0·86)	1·4 (0·61)
7	Penicillase control	2·0	0·5	3·0	2·0	2·0	1·9 (1·27)	2·8 (1·22)

Any growth observed in this experiment heavier than that
previously given the index 4·0 was recorded as 5·0.

For comparative purposes the figures shown in brackets were
calculated as multiples of the figure for the control. There is very
good agreement between the figures obtained by visual examination
and by weighing the cultures (correlation coefficient —0·98, P<0·001).

Since much the same increase of growth was obtained with the
590 unit penicillin and the pure salt, it would appear that the effect
can be ascribed to penicillin and not to impurities. This view is con-
firmed by the observation that groups 6 and 7 showed no appreciable
increase or decrease in growth.

We have recorded these observations not because they have any
bearing on the therapeutic use of penicillin, but as being of possible
interest in connection with the biology of *M. tuberculosis*.

Hand's (1946) findings, which we had the opportunity of seeing
before publication, are not in direct contradiction to ours, because
his experiments were performed under quite different conditions with
a different medium and technique of growth, shorter incubation period
and much higher doses of impure penicillin.

Summary

The addition of small amounts of penicillin to two media used for culture of *M. tuberculosis* results in an increased growth of the organism. The effect appears to be due to penicillin itself and not to impurities.

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OBSERVATIONS ON EXPERIMENTAL AMMONIUM CHLORIDE ACIDOSIS

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(PLATES LXXX-LXXXII)

A CONSIDERABLE volume of work has recently been done in an attempt to determine the cause of the very similar renal lesions found in " crush injury ", incompatible transfusion and certain cases of pyloric stenosis. The strict localisation of the tubular damage in these conditions has suggested a common cause, and one of the main theories is that expressed by Dunn, Gillespie and Niven (1941), who maintain that the necrosis is due to acidification processes taking place in the distal part of the nephron. This idea is based mainly on the similar explanations given for the necrosis resulting from the administration of uric acid (Dunn and Polson, 1926), oxalates (Dunn, Haworth and Jones, 1924) and phosphates (McFarlane, 1941). The presence of various pigments in some of the clinical conditions mentioned has led other investigators to modify this theory. Bywaters and Stead (1944-46) found that myohæmoglobin administered to rabbits excreting an acid urine caused uræmia. Similar effects produced by hæmoglobin were reported by Baker and Dodds (1925). Ammonium chloride was the substance used to induce excretion of an acid urine. It seemed desirable, therefore, to study the effect of acidosis *per se* on the kidney, using ammonium chloride for this purpose.

EXPERIMENTAL METHODS

Preliminary experiments indicated that the desired toxic dose lay somewhere between 0·5 and 1·0 g. of ammonium chloride per kilo of body weight. In all, 14 rabbits from various sources were used. These were placed in simple urine cages and fed on wet bran and oats for a stabilising period of one week. Two hundred ml. of water were used to wet the food and the animals usually consumed at least half of the food supplied daily. Urine was collected daily for the estimation of urea, chlorides, phosphates, ammonia and pH, and daily tests were made for albumin. Blood was withdrawn from an ear vein twice during this period for the estimation of urea, chlorides, phosphates, lipoids, ammonia, CO_2-combining power and pH.

At the end of a week the animals were divided into three groups. Group A (6 animals) received 0·5 g., group B (6 animals) 0·75 g. and group C (2 animals)

1·0 g. of ammonium chloride in 20 per cent. solution per kilo by stomach tube daily. Urinary and blood examinations were made as in the pre-experimental period.

Chemical methods. The urinary and blood urea values were estimated by the hypobromite and urease methods respectively. Volhard's method was used to determine the urinary and blood chlorides. Kuttner and Lichtenstein's (1930) technique was adapted for the estimation of urinary and blood phosphates. Urinary ammonia and plasma ammonia levels were found by the common aeration method. The pH of both urine and plasma was calculated electrometrically using a quinhydrone electrode. The usual van Slyke manometric method was employed to calculate the plasma CO_2-combining power. A Busch-Rückert hæmolipocrit, previously tested against the ordinary chemical extraction method, was used to estimate the plasma lipoids.

Histological methods. Material for microscopic examination was fixed in formalin and in alcohol. Sections were stained with hæmatoxylin and eosin, Masson's trichrome method, Dunn's aniline blue orange G method, Gomori's method for alkaline phosphatase, Best's carmine stain for glycogen and Sudan IV.

RESULTS

During the stabilising period the animals appeared to be healthy. The urinary volume was high and there was a slight rise in the output of urea and a more marked increase in the phosphate excretion. In most cases the urinary pH fell progressively and in every animal when the figure fell below 6 albumin appeared in the urine. Blood urea figures were at the upper limit of normal. No change could be found in the plasma chlorides, phosphates, lipoids, ammonia, CO_2-combining power or pH.

Group A. Six animals receiving 0·5 g. ammonium chloride per kilo

Animals 1–5. These animals were killed on the 5th day of the experiment: the sixth died on the 10th day. The first five animals appeared to be healthy throughout the experiment but had a decreased appetite. There was an increased output of water and urea but the phosphates were diminished. The urinary pH fell progressively, but on the 4th day it tended to rise and in two animals it passed the neutral point. Blood urea values were raised, but rarely above 70 mg. per 100 c.c. A marked fall in CO_2-combining power of the plasma occurred and the plasma pH was slightly reduced. Little change could be detected in the blood phosphorus or ammonia levels. The plasma lipoids were around 200 mg. per 100 c.c. There was of course some increase in blood chlorides. Table I gives the more striking average values before and after administration of ammonium chloride. Some of the detailed changes in animal 4 are shown in fig. 1.

All animals were killed on the 5th day of the experimental period by a blow on the back of the neck. The heart appeared to be normal but the lungs showed occasional depressed and congested areas of collapse and a few pleural petechiæ. There was some pallor of the

TABLE I

Animals (5) receiving 0·5 g. ammonium chloride per kilo

No	Urine (volume)		Urea output		Phosphate output		Blood urea		CO₂-combining power	
	1	2	1	2	1	2	1	2	1	2
1	134	105	1·01	1 8	230	105	35	72	35 0	15
2	103	130	1·27	2 3	180	87	37	88·	36·0	14
3	86	43	2 5	1·4	288	79	40	66	38·5	13
4	38	54	1·35	1 8	156	128	38	64	37 0	14
5	62	09	1·1	1 6	109	95	40	52	36·5	15

1 = before, 2 = after administration of ammonium chloride.
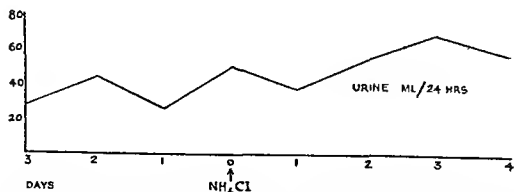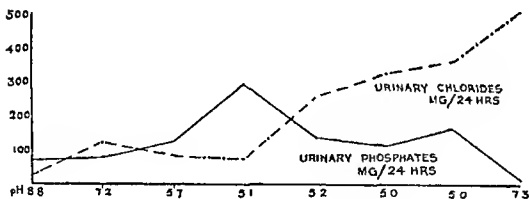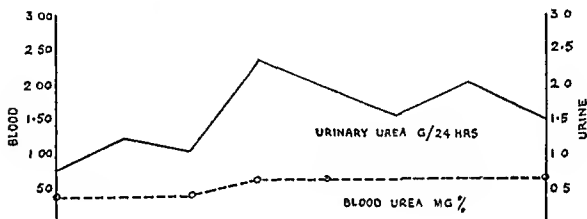
FIG. 1.—Graphs illustrating the effect on animal 4 of administering 0 5 g. ammonium chloride per kilo for 4 days. The animal was killed on the 5th day.

liver but no change could be seen in the spleen. The kidneys were rather swollen and congested and the cut surface showed that the congestion was most marked in the outer cortex and medulla. At the junction of inner cortex and outer medulla there was a faint zone of pallor. No significant change could be seen in the brain.

Histology (animals 1-5). Material was taken from the kidneys, lungs, liver, brain and spleen and both paraffin and frozen sections were prepared. *Lungs.* Areas of patchy collapse were present and in association with most of these were numerous fat emboli. A few tiny infarets were present in rabbit 4, but they were absent in the other animals. The bronchi in all animals appeared to be irregularly dilated. *Liver.* In two animals (1 and 2) there was a hydropic change in some of the liver cells. The cytoplasm was flocculated into a few large cloudy granules and the cell boundaries were unusually distinct. There was no sign of fatty degeneration. In the other animals the liver was normal. *Spleen.* Apart from some congestion of the pulp this organ was normal. *Brain.* In one animal there were occasional small fat emboli in the cortical vessels. No sign of softening could be seen. *Kidneys.* The cortical veins were widely distended. Marked dilatation of the glomerular capillaries was also present but no pathological change could be seen in this region. The proximal convoluted tubules showed marked catarrh and the cells were somewhat vacuolated. There was pronounced dilatation of the remainder of the nephron including the narrow limbs of Henle. The cells of the broad ascending limbs and collecting tubules were flattened and in a few of the ascending limbs occasional necrotic cells with pyknotic nuclei could be seen. There was no sign of cast formation apart from occasional traces of albuminoid material obviously arising from degeneration of the tubular epithelium.

Animal 6. The sixth animal of this group began to lose condition about the 6th day of ammonium chloride administration. From the 5th day its appetite diminished progressively and during the last 72 hours of life it starved itself voluntarily. Prior to the 5th day its appetite had been increased. It lost weight and on the 10th day it became drowsy, showed a tendency to fall over and soon became comatose and died. Despite the starvation the urinary volume was maintained except on the 5th day, when it failed to pass urine. The urea output followed the water excretion fairly closely. An increase of phosphate output occurred in the first few days but it tended to decrease later. Urinary ammonia was increased to 25 mg. per day but the pH remained low till the end. There was increased blood urea, which just before death reached 120 mg. per 100 c.c. The CO_2-combining power of the plasma was reduced to 14·5 volumes per cent. and the plasma pH to 6·92. Plasma lipoids were increased to 400 mg. and ammonia to 1·29 mg. per 100 c.c. Fig. 2 gives a picture for comparison with animal no. 4 (fig. 1).

Post-mortem appearances were very similar to those seen in the

other animals of this group. The renal changes were rather more striking. The faint zone of pallor noted before was more pronounced and tended to bulge from the surface as a distinct band.

Histology. Lungs. The changes were similar to those in the other members of group A. Areas of collapse with surrounding compensatory emphysema were common. Fat emboli were fairly numerous and large. Many of the vessels were thrombosed and others contained immense numbers of polymorphonuclear leucocytes, a large proportion
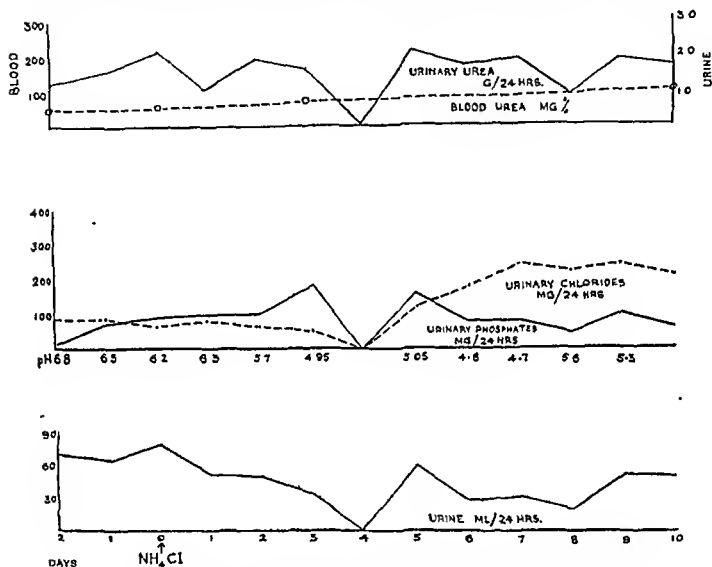
Fig. 2.—Graphs illustrating the effect on animal 6 of administering 0·5 g. ammonium chloride per kilo for 9 days. This animal died on the 10th day.

of which were eosinophils. *Liver.* In some of the lobules the cells showed pyknotic changes, most marked in the central zones. The remainder of the hepatic tissue appeared to be normal. *Spleen.* The sinusoids were dilated and congested. Pigmented phagocytes were present in considerable numbers. *Brain.* Small softenings were present in many parts of the brain, with thrombosis of the associated vessels. No signs of fat emboli could be seen. *Kidneys.* The glomeruli were uniformly congested but otherwise normal. The first convoluted tubules showed vacuolation of the epithelium and in a few cells the nuclei were undergoing karyolytic changes. More marked degeneration was noted in the ascending limbs, the broad segments of which

showed striking disintegration of the cytoplasm, with pyknotic and karyolytic degeneration of the nuclei. Large casts staining strongly with eosin were found in many of the second convoluted tubules and in these the normal epithelium was replaced by a thin layer of cells with hyperchromatic nuclei.

Group B. Six animals receiving 0·75 g. ammonium chloride per kilo

Four died on the 4th day of the experimental period, a fifth animal (no. 11) was moribund and was killed on the same day by a light blow on the back of the neck. The sixth animal died on the 5th day. All animals exhibited similar signs. A few hours after the last dose of ammonium chloride they became stuporose. The fore limbs tended to splay out and eventually became flaccid. Their heads turned to one side, and they repeatedly fell over to the same side. Breathing was irregular and deep. Two animals had minor convulsions but the others lay quietly. They finally became comatose and died within sixteen hours. During the experimental period their appetite was diminished. The output of urine was maintained and little change could be seen in the urea excretion, which was high. The phosphate excretion was diminished. There was a fall in urinary pH but this was not so marked as in group A, and towards the end there was a distinct rise, in most cases above the neutral point. In all animals the blood urea rose and the final figure varied between 120 and 170 mg. per 100 c.c. The plasma CO_2-combining power showed a greater diminution than in group A, usually to 10 or 11 volumes per cent., and the plasma pH fell below 7. No change could be found in the plasma phosphorus but the ammonia rose to very high levels, reaching 14 mg. per 100 c.c. in one case. Plasma lipoids were increased, the figure ranging from 500 to 600 mg. per 100 c.c. Blood chlorides were high, almost 700 mg. Table II gives the average values of the more important constituents before and after administration of ammonium chloride. Fig. 3 shows the detailed changes in no. 9.

TABLE II

Animals (6) receiving 0·75 g. ammonium chloride per kilo

No.	Urine (volume)		Urinary urea		Urinary phosphate		Blood urea		Plasma CO_2-combining power	
	1	2	1	2	1	2	1	2	1	2
7	140	130	1·6	1·9	160·0	72·0	43	173	38·0	11·3
8	110	105	1·5	2·7	180·0	85·0	42	165	37·0	12·2
9	89	65	1·1	1·1	92·0	60·0	46	169	36·0	10·5
10	74	49	1·7	1·3	104·0	55·0	47	171	37·5	11·0
11	94	94	1·4	1·7	176·0	98·0	45	170	35·0	10·0
12	75	64	1·7	2·0	126·3	162·6	45	112	38·0	10·0

1 = before, 2 = after administration of ammonium chloride.

The changes *post mortem* were very similar to those observed in group A. The lungs showed patches of collapse surrounded by areas of emphysema. No abnormality could be seen in the heart. There was some pallor of the liver but the spleen appeared to be normal. In the kidneys the narrow zone of pallor observed in the outer medulla
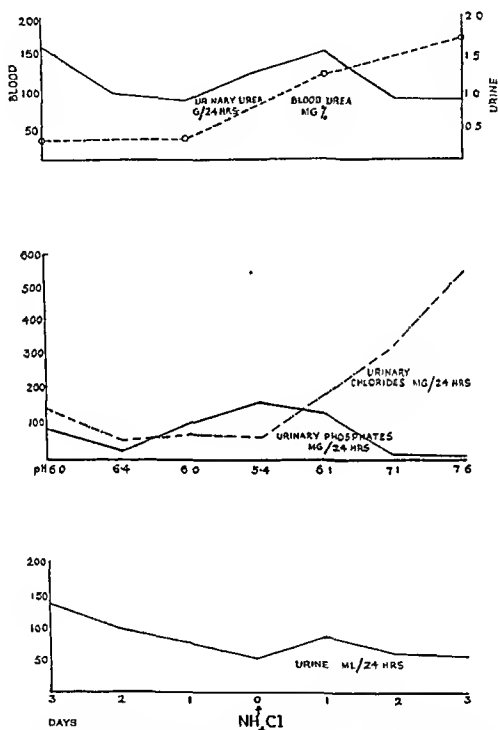
Fig 3 —Graphs illustrating the effect on animal 9 of administering 0 75 g ammonium chloride per kilo for 3 days The animal died on the 4th day.

in group A was very marked (fig. 4) and was much broader. The remainder of the renal substance was much congested. No particular change could be seen in the brain except in no. 11. In this animal there was extensive subarachnoid hæmorrhage, presumably due to the blow on the back of the neck.

Histology. Lungs. Areas of collapse with many fat emboli were

common (fig. 5) and there was emphysema of the adjacent lung substance. Small masses of fat could also be seen in some of the peribronchial lymph nodes. The bronchi showed irregular dilatation. *Liver.* All the vessels were congested, especially the central veins. Hydropic degeneration of the liver cells was marked (fig. 6). *Brain.* A few tiny softenings were present in the cerebrum but there was no evidence that fat embolism was the cause. Some of the capillaries were thrombosed. In no. 11 the surface vessels were much congested but, apart from this, no evidence of the cause of the subarachnoid hæmorrhage could be seen. *Kidneys.* There was marked congestion of the outer cortex and outer medulla, but between them, in the zone containing the broad ascending limbs of Henle, the vessels were empty. The glomeruli were congested but appeared to be otherwise normal. Catarrhal changes were present in the first part of the proximal convoluted tubule but no gross change could be seen until the lower part of the straight portion of this tubule was reached. Here most of the cells were greatly swollen and in process of disintegration and their nuclei showed karyolysis. The narrow limbs of Henle were dilated and also showed necrosis in many parts. Advanced necrotic changes were present in the broad ascending limbs. In many instances the epithelial cells had disappeared, while in others pyknotic nuclei indicated an earlier phase (figs. 7 and 8). A few showed polymorph infiltration of the basement membrane. In some of the collecting tubules and ducts of Bellini there were necrotic epithelial cells, whilst others contained colloid casts.

Group C. Two animals receiving 1·0 *g. ammonium chloride per kilo*

Both these animals died less than sixteen hours later, and owing to the short interval no significant figures are available for the biochemical changes.

The post-mortem and histological changes were exactly similar to those found in group B, except that in the kidney the necrosis was confined to the broad ascending limbs of Henle (fig. 9).

Discussion

There are few relevant references in the literature to the pathological action of ammonium chloride. Markert (1933) records experiments on healthy human subjects and on those with spontaneous renal lesions. Healthy subjects received 0·1-0·2 g. per kilo daily for 5-8 days. Acidosis developed, the urinary acidity rose and the CO_2-combining power of the plasma fell. There was an increased output of ammonia, urea and chloride. Albuminuria and hæmaturia appeared and the urine contained casts. Haldane (1921) made experiments on himself by taking ammonium chloride, but the dose was smaller than that administered by Markert and although acidosis developed there was apparently no albuminuria.

PLATE LXXX

AMMONIUM CHLORIDE ACIDOSIS

Fig 4 —Kidney of animal 9, group B   The pale zone at the junction of cortex and medulla is clearly shown
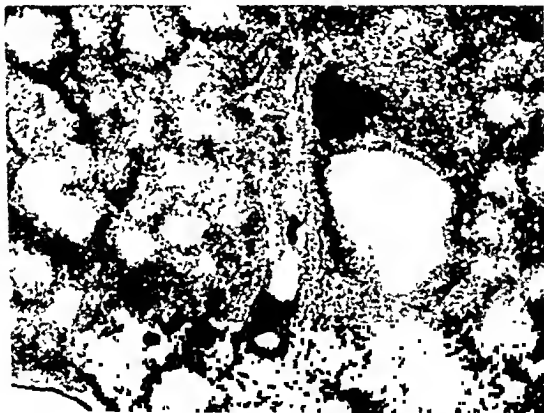
Fig 5 —Section of lung from animal 10, group B, showing a large mass of fat in one of the pulmonary vessels   Hæmatoxylin and Sudan IV   ×90

AMMONIUM CHLORIDE ACIDOSIS
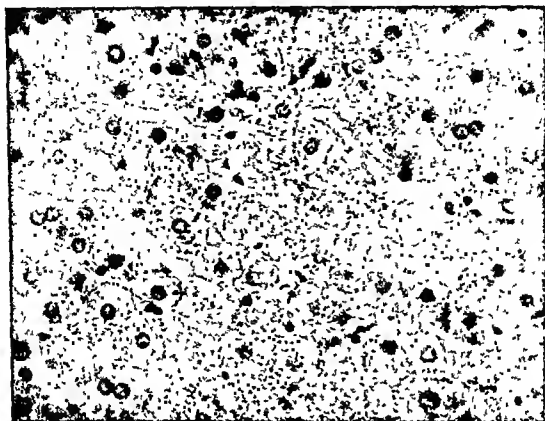
FIG. 6.—Section of liver of animal 7, group B, showing clumping of cytoplasm into coarse granules (hydropic degeneration) and increased definition of cell membranes. H. and E. × 100.
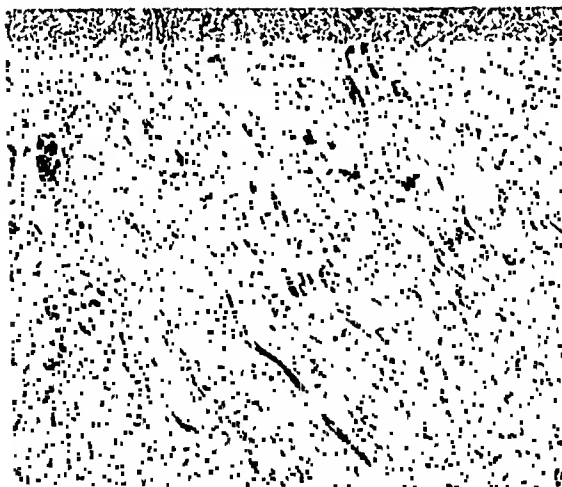
FIG. 7.—Section of kidney from animal 8, group B, showing diffuse degenerative changes in the ascending and descending tubules. H. and E.   × 100.

Before discussing the results of the present experiment it is obviously necessary to separate the results in the five animals of group A from the remainder. Most of the chemical changes in these five animals are similar to those seen after therapeutic doses of ammonium chloride. For example the urinary volume and urea output are increased. The decrease in phosphate excretion appears to be related to the decreased intake of food. This is suggested by comparison of the results in these five animals with those of the sixth animal, which had an increased appetite and an increased output of phosphate in the first few days of the experiment. The blood urea figures are raised, but since there is practically no renal damage this increase may well be due to conversion of the ammonia radicle of the ingested salt to urea.

The chemical and pathological changes in animal 6 are very similar to those found in group B, but it is more than likely that its death was hastened by starvation. In group B the blood urea is markedly increased and this, combined with the tubular necrosis in the kidneys, suggests that the death of these animals is directly due to nephritis, though the biochemical changes are not typical. The highest blood urea figure is 173 mg. per 100 c.c., and while this is certainly a notable increase, it is not comparable with figures obtained with other nephropathic agents. Dunn and Polson in their studies of uric acid nephritis record anuria or oliguria in their animals, with blood urea figures ranging from 300 to 600 mg. per 100 c.c. Similar results were found by Dunn, Howarth and Jones in experimental oxalate nephritis. The relatively low blood urea values in the present series might be due to failure of the liver to complete the deamination process, but it is significant that the urinary volume and urea output were maintained at a high level. Dunn and Polson were of the opinion that the high blood urea and oliguria in experimental nephrosis were due to increased reabsorption from the damaged tubules.

One of two conclusions might be drawn from the present experiments: either (a) ammonium chloride in some way increases the output of fluid, which washes out the urea, or (b) Dunn and Polson's conclusions are wrong, and in other experimental nephroses the glomerular filtrate is reduced. Dunn and Jones (1925), however, have shown that, in oxalate nephritis at least, an increased fluid intake will diminish the blood urea by increasing the renal excretion. It seems reasonable to suggest that in the present series of experiments the relatively low blood urea figures are due, in part at least, to maintenance of urinary output and that this is probably associated with the high chloride excretion.

The cause of the renal lesion would appear to be acidosis. An acid urine is an abnormality in the rabbit and in the present experiment a trace of albumin appeared in the urine whenever the pH fell below 6. This necessarily means renal dysfunction, and results obtained by injecting myohæmoglobin (Bywaters and Stead) and

hæmoglobin (Baker and Dodds) after administering ammonium chloride cannot be entirely attributed to the pigment concerned. It is surprising that this reaction of the rabbit kidney to mild acidosis has not been noted before. Apart from this, the above experiments seem to show that the distal nephron of the rabbit kidney is adversely affected by acid substances, but it is curious that the urinary pH in group A is frequently lower than that in group B and yet the lesions are more marked in the group B animals.

Necrosis due to a transient change in pH followed by an inability to excrete acid might be a possible explanation and it is significant that the urinary pH rose in animals of group B two or three days before death, although the blood acidosis continued. One other possibility may be considered. Estimation of the plasma ammonia shows that it is greatly increased in group B, reaching 14 mg. per 100 c.c. in one animal. Ammonia is notoriously toxic in the blood and is usually rapidly converted into urea. Much of the accumulated ammonia may have come from the ingested salt and, since Svedberg, Maddock and Drury (1938) have shown that in the rabbit the liver is the sole source of urea, it would suggest either that the rabbit liver is unable to deal with such large quantities of ammonia or that the liver is damaged by the acidosis. In either case there is evidence of a considerable degree of ammonia intoxication and this may be the cause of the renal lesions. There is no direct evidence of this, however, whereas it has been shown that lowering the pH' of the urine by administering an acid-producing diet injures the kidney and causes albuminuria.

Finally, it is remarkable to find fat " emboli " in acidosis. There have been reports from time to time of apparent intravascular formation of fat emboli, among them that of Ogilvie (1932), who found them in the kidneys and lungs in experimental mercury nephritis. Two factors seem to us to be especially significant in this connection. First, there seems no reason to doubt that the fat of the emboli is derived from the plasma fat, which is increased in amount. Secondly, the emboli are found in the lungs in our animals and in the kidneys and lungs in Ogilvie's experiments. These organs are the site of the most marked fluctuations in pH and it seems reasonable to suggest that the emboli are formed *in situ* by flocculation of the plasma fat at some particular hydrogen ion concentration. These accumulations of fat might be better termed fat " thrombi ". It is important to note that the reaction of the lungs to the resulting vascular obstruction is collapse and not infarction.

SUMMARY

1. The administration to rabbits of 0·5-1·0 g. ammonium chloride per kilo causes necrosis of renal tubular epithelium with subsequent death of the animal.

AMMONIUM CHLORIDE ACIDOSIS
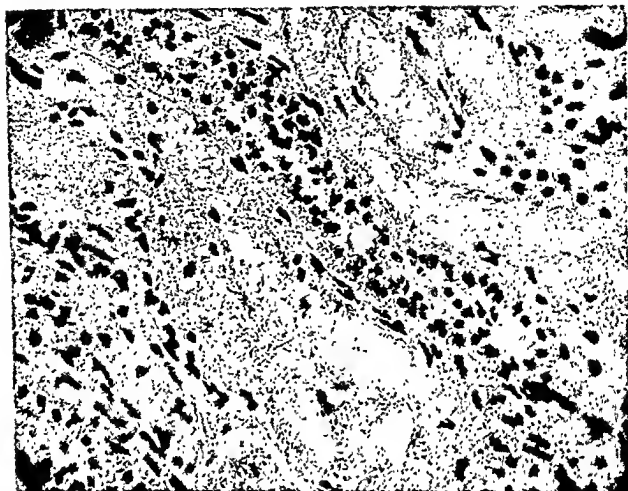
FIG. 8.—High-power view of broad ascending limbs of Henle in animal 8. Note advanced necrosis. H. and E.   ×450.
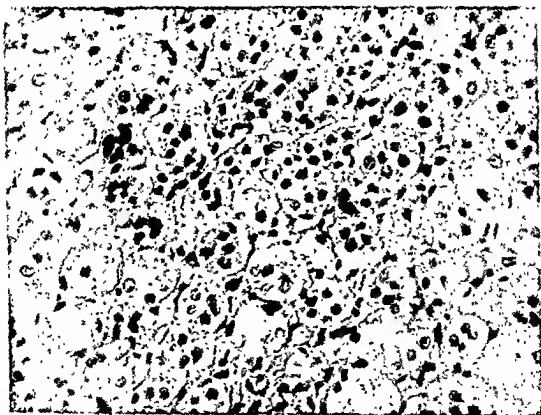
FIG. 9.—Cross section of ascending limbs of Henle in kidney from animal 13, group C. Many of the cells show pyknotic nuclei and in others there is karyolysis and fragmentation of the cytoplasm. H. and E.   ×390.

2. The necrosis is found mainly in the distal portion of the rabbit nephron, but there is evidence to show that the proximal part is also affected.

3. Fat emboli have been observed in the lungs of animals dying after ammonium chloride administration.

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DISSEMINATED PARENCHYMATOUS OSSIFICATION IN THE LUNGS IN ASSOCIATION WITH MITRAL STENOSIS

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(PLATES LXXXIII-LXXXV)

SINCE Aschoff bodies have been recognised as a specific lesion, it has been shown that rheumatic manifestations are not confined to the heart and joints only, but that submiliary rheumatic nodules can be found in fibrous tissue in various parts of the body. Aschoff (1904) had already observed these rheumatic nodules to be situated in the neighbourhood of the smaller and medium sized vessels and in close relationship to their adventitia. He often found the vascular walls to be affected, the lesion being comparable to that of polyarteritis nodosa. Later investigations by VonGlahn and Pappenheimer (1926), Paul (1927), Karsner and Bayless (1933-34) and others have shown the frequent involvement of the coronary and pulmonary arteries and their branches. According to Boyd (1944), rheumatic aortitis occurs in most cases of rheumatic heart disease and Pappenheimer and VonGlahn (1923-24) found, in long-standing cases of rheumatic valvular disease, well marked scars in the aorta near the nutrient vessels.

Typical Aschoff bodies were described in the lungs by Fraser (1930) and Gouley (1938), an observation not confirmed by other investigators. These divergent findings may perhaps be explained by the difficulty in detecting Aschoff bodies or similar lesions in a loosely knit tissue like the lung and in particular during the stages of consolidation and widespread mononuclear infiltration in rheumatic pneumonia. In the chronic stage, however, when rheumatic consolidation is absent, lesions resembling Aschoff nodes may be found in the lungs. Hadfield (1938) made a detailed study of the macroscopic and histological changes in the rheumatic lung and found the primary changes to be strictly confined to the regions of gaseous exchange. The characteristic features are a fibrinous, almost acellular alveolitis, followed some days later by infiltration of mononuclear cells. The alveolar ducts are wide open and prominent, and lined by a hyaline eosinophil membrane. Most of the alveolar ducts contain an exudate which is

probably albuminous in composition, while the infiltration by mono-nuclear cells constitutes a mesenchymal phagocytic reaction. The exudate in the alveolar ducts, in undergoing fibroblastic replacement, results in what has been regarded as interstitial fibrosis.

From these observations it may be concluded that rheumatic manifestations occur in the lungs which are at least characteristic if not specific. These changes are not directly dependent on mitral stenosis or passive congestion, since they have been observed also in the acute and subacute stages of rheumatic fever (Gouley and Eiman, 1932 ; Masson, Riopelle and Martin, 1937 ; Epstein and Greenspan, 1941 ; Neubuerger, Geever and Rutledge, 1944).

In recent years a few isolated cases of long-standing mitral stenosis have been reported in which roentgenograms of the chest showed disseminated calcified pulmonary nodules. These on post-mortem examination proved to consist of bone and showed no evidence of tuberculous infection. The first description correlating the two conditions was given by Salinger (1932). Since then several reports in Continental and American literature have appeared (Janker, 1936 ; Gross, 1938 ; Manzini, 1938 ; Munk, 1939 ; Wells and Dunlap, 1943, Grishman and Kane, 1945). As far as we can ascertain no such case has as yet been reported in this country, but as Grishman and Kane have been able to publish no fewer than 8 personal cases the condition does not appear to be so rare as the paucity of the case reports would seem to indicate.

A typical example of this remarkable association has been observed by us and in the following account the radiological diagnosis of the pulmonary lesions and some aspects of their possible pathogenesis will be discussed.

Case report

T. W., male, 32 years, had " growing pains " when 16 years of age and at that time valvular disease of the heart was diagnosed. Clinically the patient showed the typical picture of mitral stenosis with auricular fibrillation and cardiac failure. Roentgenoscopic and roentgenographic examination of the chest on 20.2.44 confirmed the clinical diagnosis of mitral stenosis with advanced passive pulmonary congestion. Besides these common radiological findings numerous densely opaque nodules of pinhead to small pea size were observed in both lung fields. The lesions were predominant in the right lower zone, particularly in its periphery, and less numerous in the right middle zone and subclavicular region. Similar lesions were present in the left lung but were partly obscured by the gross enlargement of the heart. The nodules were well defined and the larger were mulberry-like in shape. The apices were not involved. There was widespread fibrosis and compensatory emphysema in both lungs (figs. 1 and 2).

Summary of autopsy findings

A well built, well nourished young man ; no external markings or scars ; moderately jaundiced ; slight pitting oedema of both legs ; rigor mortis present ; moderate hypostatic congestion.

Heart (570 g.) considerably enlarged ; both ventricles hypertrophied, especially the right ; both auricles dilated and hypertrophied. Pericardium.

PULMONARY OSSIFICATION

FIG. 2.—The right lower lung field of fig. 1 at a higher magnification.

FIG. 1.—Radiograph of chest showing mitral configuration of the heart with hilar congestion of the lungs, and widespread nodular densities predominantly in the right lower lung field

myocardium and endocardium normal. Mitral cusps thickened and fused, with large calcified nodular masses on their auricular surface; chordas much thickened; valve orifices stenosed. Aortic cusps thickened, slightly calcified and slightly incompetent. Tricuspid ring dilated and valve apparently incompetent. Coronary arteries and aorta only slightly atheromatous; aorta relatively narrow.

Lungs (rt. 1240 g., lt. 920 g.). Approximately 200 ml. of cloudy brownish fluid in each pleural cavity. Dense fibrous adhesions over most of left upper lobe; scattered adhesions over right lung. Severe brown induration of both lungs; two small infarcts in right lower lobs and one in left costophrenic region; thrombus in corresponding arteries. Frothy fluid in air passages. Innumerable calcified nodules 2·5 mm. in diameter scattered throughout both lungs but predominantly in the lower lobes. A few calcified nodules in the visceral pleura. Hilar glands swollen and œdematous but not calcified.

Liver (1550 g.) very firm and tough; surface finely granular, lobular pattern accentuated by passive congestion; slightly bile stained. *Gall bladder* distended with dark fluid bile. Ducts and pancreas normal. *Spleen* (270 g.) swollen, firm and congested. *Kidneys* swollen, firm and congested; pelves, ureters, bladder and prostate normal. *Alimentary canal* normal; *peritoneum* normal. *Thyroid* and *adrenals* normal.

Microscopical examination

The spleen and kidneys show severe passive congestion. The liver, in addition to centrilobular congestion, has developed a considerable increase of poorly cellular fibrous tissue in both centrilobular and periportal areas. The extension of the fibrous tissue of the portal tracts has produced a certain degree of perilobular cirrhosis. No significant changes are observed in the myocardium.

The lungs are passively congested, the alveolar walls thickened by capillary engorgement and an increase of mononuclear cells. Several alveoli and groups of alveoli are consolidated by closely packed " heart failure " cells rich in hæmosiderin. A well marked interstitial fibrosis is present but only in patches (fig. 3). Severe degenerative and inflammatory changes are present in the smaller pulmonary vessels. In arteries 0·1-2·0 mm. in external diameter, two types of change are seen, (1) small atheromatous plaques composed of foamy cells, and (2) severe fibrinoid medial necrosis with destruction of the elastica, including the internal elastic lamina. The intima corresponding to the areas of medial necrosis is remarkably thickened by fibrocellular tissue containing capillary spaces, and the adventitia is moderately infiltrated with lymphocytes and polymorphs (fig. 4). The vasa vasorum in the adventitia of the larger of these small arteries have also undergone some degree of obliterative endarteritis. In none of these severely injured vessels do the changes appear to be secondary to thrombosis and no hæmosiderin is detected in the intimal granulation tissue. In fact, the only thrombi found are in apparently healthy pulmonary vessels. Throughout the lungs several of the arterioles of about 100 μ external diameter show " onion skin " thickening of the wall resulting from endothelial cell proliferation.

The bony particles within the lungs are of the woven type and all

except the largest are free from marrow spaces. The bone is nowhere interstitial in position but fills one alveolus or a group of alveolar spaces and their corresponding alveolar duct (fig. 5). That the framework of the lung is incorporated in the developing bone is clearly demonstrated by elastic staining, which shows the continuity of the elastic tissue of the lung with that in the bone (fig. 6). No iron is present in the osseous tissue.

Apart from typical bone, intra-alveolar acellular material resembling osseous ground substance is also present in some areas (fig. 7). That this peculiar intra-alveolar material constitutes the precursor of the true bone found elsewhere in the lung is suggested by the presence of similar material forming a cap to some of the projections of the bony structures themselves.

DISCUSSION

The above-described pulmonary changes in mitral stenosis are of practical importance. Pulmonary tuberculosis is a common complication of congenital heart disease, but is only rarely met with in mitral stenosis. It would seem likely that in at least some cases of mitral stenosis miliary shadows in the lungs have been wrongly interpreted as due to tuberculosis, when in fact they were caused by the so-called miliary type of pulmonary congestion in mitral stenosis and particularly by the above-described osseous lesions. There is evidence in the literature of such cases having been sent unnecessarily for prolonged sanatorium treatment.

Disseminated more or less opaque nodules in the lungs may occur in silicosis, sarcoidosis, the leukæmias, Hodgkin's disease and lymphangitis carcinomatosa. Other widespread non-tuberculous nodular densities may result from fungus infections and recently Palmer (1945) has pointed out that infection with *Histoplasma capsulatum* may be a cause of pulmonary calcification. A similar X-ray picture may also be produced by " pulmonary hæmosiderosis ", in which the lung tissues contain a large amount of the radiopaque hæmosiderin—a condition so far observed in children only (Anspach, 1939).

The history, clinical course and various examinations in our case produced no evidence that any of these conditions was responsible for the pulmonary changes. Tuberculosis, the most common cause of pulmonary calcification, was the only remaining condition to be seriously considered in the differential diagnosis, and indeed the radiographic appearance of the lungs showed great resemblance to calcified miliary tuberculosis. The principal difference lies, however, in the distribution of the nodules. In chronic miliary tuberculosis the lesions are more or less uniformly spread over both lung fields, including the apices, irrespective of whether the primary lesion is intra- or extrapulmonary. In our case and in those previously reported in the literature the pulmonary nodules are situated predominantly

PULMONARY OSSIFICATION
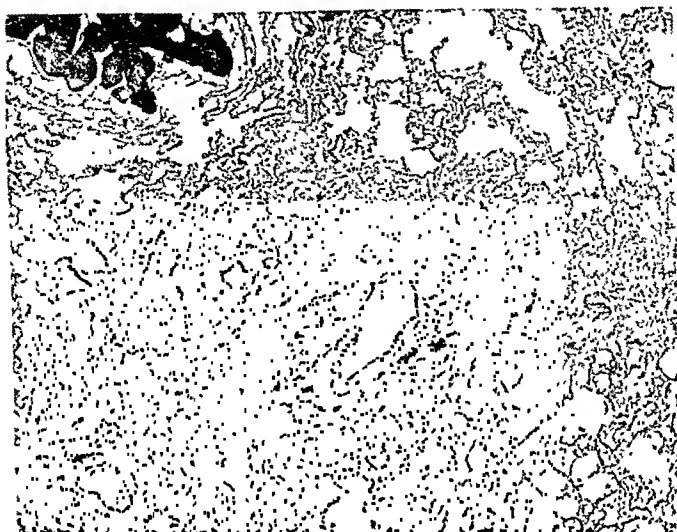
FIG. 3.—Section of lung showing a patch of interstitial fibrosis. Weigert's hæmatoxylin and van Gieson. × 28.
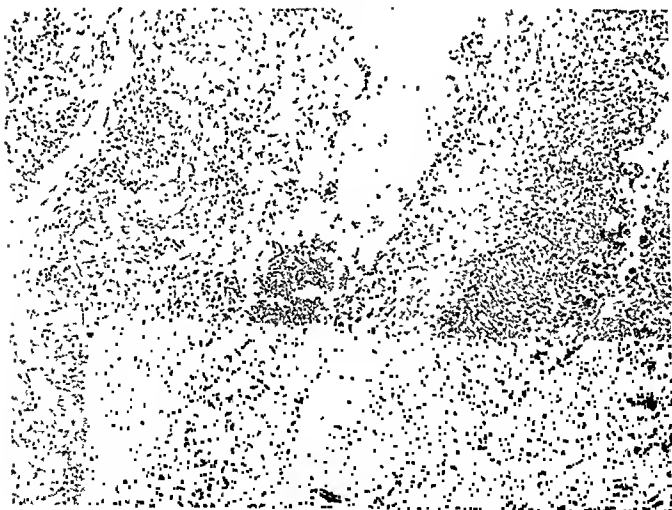
FIG. 4.—A small artery in longitudinal section, showing fibrinoid medial necrosis, considerable intimal thickening and some histiocyte adventitial infiltration. H. and E. × 90.

in the lower lung fields, becoming less numerous in their extension upwards and the apices themselves are not involved. No evidence of any active or inactive parent lesion could be detected. A radiological diagnosis of non-tuberculous calcified nodules in mitral stenosis was made.

So far there is no satisfactory explanation of the genesis of these bony nodules. Bone formation in the lungs has often been found in healed tuberculous lesions. Branching spicules of bone in the fibrous framework of the lung are occasionally met with, but almost exclusively in old men. Daust (1929) considers this to be the result of metaplasia secondary to senile changes in the perivascular connective tissue. Circumscribed intra-alveolar bony nodules have been found only in relatively young people with mitral stenosis. Previous authors have attempted to correlate their occurrence with the long-standing passive congestion of the lungs in these cases. This explanation is not convincing, since cases have been observed in which no clinical or radiological evidence of pulmonary congestion was present (Gross ; Grishman and Kane). Moreover, no cases of ossified nodules in the lungs have been reported in chronic passive congestion due to other causes than rheumatic valvular disease. This emphasises the relationship of the bony lesions to rheumatic fever. The following additional observations may be adduced in support of this conception.

In Wells and Dunlap's case, and especially in our own, intra-pulmonary vascular changes were present which have been described as characteristic of rheumatic arteritis. Patches of interstitial fibrosis with loss of elastic fibrils seen in our case indicate previous interstitial pneumonia, and are a common finding in rheumatic pneumonia. According to Hadfield the specific rheumatic lesion in the lungs is a peculiar fibrinous and acellular exudation into the alveolar ducts and associated alveoli. The bony lesions found in this type of pulmonary ossification involve the same characteristic lung unit. It is therefore suggested that nodular pulmonary ossification associated with mitral stenosis may be the late result of specific rheumatic pneumonia. The preservation of the elastic framework of the lung within the bony structure suggests that the process of ossification arises by organisation of intra-alveolar exudate. Since ossification has not been described as a feature of ordinary organised pneumonia, it is suggested that there is a specific quality in the exudate in these cases, which finds confirmation in Hadfield's description of the peculiar nature of the exudate in the acute and subacute stages of rheumatic pneumonia.

SUMMARY

1. Attention is directed to the occurrence of disseminated parenchymatous ossification in the lungs in mitral stenosis. The X-ray appearances seem to be characteristic, since the cases reported in the literature as well as our own case show the nodular opacities to be

situated predominantly in the lower lung fields and not to involve the apices.

2. Histological examination of the lungs shows that the nodular opacities consist of woven bone. The intra-alveolar distribution of the bone and the preservation of the elastic tissue within the osseous foci are emphasised.

3. Rheumatic manifestations in the lungs have also been found, and it is suggested that pulmonary ossification in mitral stenosis is not, as hitherto believed, the product of long-standing passive congestion but the late result of rheumatic pneumonia.

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PLATE LXXXV

PULMONARY OSSIFICATION

FIG. 5.—A nodule of intra-alveolar pulmonary ossification. H. and E. ×25.

FIG. 7.—Amorphous calcified material almost filling a lung alveolus. H. and E. ×330.
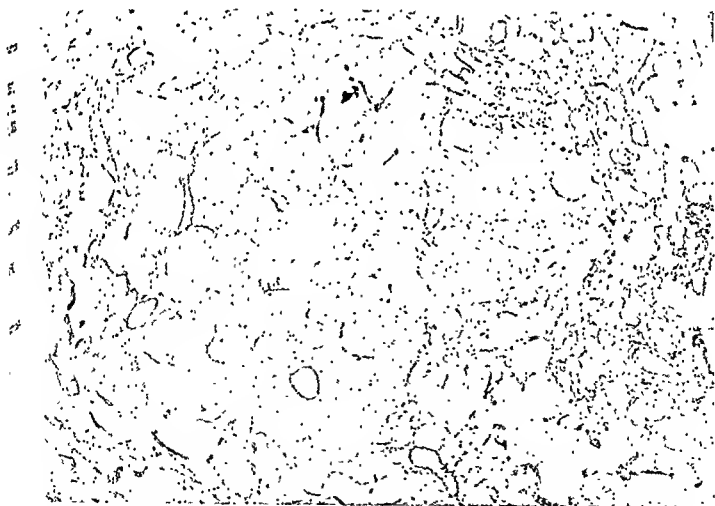
FIG. 6.—The same nodule stained for elastic fibres to show the preservation of the inter-alveolar elastic fibres within the bone substance. Weigert's elastic stain. ×40.

BIOCHEMICAL INVESTIGATIONS IN LIVER DISEASE : SOME CORRELATIONS WITH HEPATIC HISTOLOGY

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(PLATES LXXXVI-LXXXVIII)

THE use of biochemical methods in the clinical investigation of liver disease is indicated for two purposes, (1) to arrive at an accurate diagnosis and (2) to estimate the severity of the liver damage. The rationale for the various methods employed has hitherto been based on evidence provided by animal experiment or on records of cases of liver disease which have been verified at operation or necropsy. Assessment solely by clinical methods is often fallacious. An important approach to the evaluation of liver function tests is by comparing the results obtained with the histological picture in the liver. The appearance of the liver cells in section cannot of course be identified with the vital functions of these units. Visible changes in the cell do not represent specific disturbances of function. However, until the establishment of a method capable of directly estimating the workings of human liver cells *in vitro*, morbid histology would seem to be a reasonable index to the value of a liver function test. In this paper, liver diseases have been divided into four main groups :— acute hepatitis, cirrhosis, obstructive jaundice and miscellaneous, and the histological picture of the liver is compared with the results of some commonly used biochemical investigations.

METHODS

Liver tissue was obtained by a modification of the aspiration liver biopsy technique of Iversen and Roholm (1939). The method, its risks and applicability, have been described elsewhere (Sherlock, 1945). In a few instances, material obtained at necropsy or operation was used. These latter sources may be unreliable. Even before death glycogen usually disappears from the liver cells, and post-mortem autolytic changes proceed rapidly (van Beok and Haex, 1943). Necropsy often takes place some time after the performance of the liver function test. Material obtained by surgical exposure of the liver is subject to the effects of trauma and anaesthesia. Moreover, the interpretation of histological changes in small samples from the extreme liver edge is often difficult. Aspiration liver biopsy can be done in close time-relation to the liver function test

and fixation of the material is immediate. In some cases the progress of the disease was studied by comparison of sections obtained by serial biopsies with serial biochemical studies.

Biochemical methods

The estimations carried out were the serum bilirubin, alkaline phosphatase and total serum cholesterol, together with the total and differential serum proteins. The intravenous test of hippuric acid synthesis and the intravenous galactose test were also done. Obviously, all these investigations and the liver biopsy cannot be performed on the same day : however, it is possible to complete them all in three days. All measurements of time have been reckoned from the second day of investigation, on which the biopsy was always made.

Serum bilirubin was estimated by the method of Haslewood and King (1937). The upper limit of serum bilirubin in normal subjects was taken as 1 mg./100 ml.

Serum alkaline phosphatase was determined by the method of King and Armstrong (1934). The normal range is 3·7-13·1 units/100 ml. Most results lie between 5 and 10 units, and 10 units was taken as the upper limit of normal.

Serum cholesterol was estimated by a modified Liebermann-Burchard reaction (Sackett, 1925). Normal serum cholesterol is 120-230 mg./100 ml.

Serum proteins. Total serum protein, serum albumin and serum globulin were estimated by a nesslerisation method (King *et al.*, 1937, 1942). The normal ranges are :—total serum proteins 6-8 g., serum albumin 3·4-5·0 g., serum globulin 1·5-3·0 g./100 ml., and the albumin-globulin ratio 1·3/4.

Intravenous galactose tolerance test. Galactose was prepared and sterilised according to the technique of King, Harrison and Delory (1940) : 0·5 g. (1 ml. of a 50 per cent. solution) per kg. body weight was injected intravenously into the fasting subject. No toxic effects occurred. The samples, consisting of 0·2 ml. of capillary blood, were washed into the requisite amounts of isotonic sodium sulphate and sodium tungstate. They were taken before injection, and at $\frac{1}{2}$, 1, $1\frac{1}{2}$ and 2 hours afterwards. Analysis was by the method of King and Aitken (1940). In 40 out of 41 subjects without liver disease the galactose had disappeared from the blood within two hours. This 2-hour elimination is a suitable normal standard for general clinical use but, as it makes no distinction between the various types of normal curve, for purposes of comparison a modification of the " galactose time " introduced by Barnes and King (1943) was adopted to express the difference between normal and pathological tests.

$$\text{Galactose time} = \frac{ax}{(a-b)}$$

where a = 30 min. value for blood galactose,
$\quad\quad b$ = last figure for blood galactose before complete elimination from the blood, and
$\quad\quad x$ = 30, 60 or 90 minutes, according to whether b is the 60, 90 or 120 minute blood galactose value.

The galactose time expresses the rate of disappearance of galactose from the blood in minutes. It approximates to an average rate of removal of galactose from the blood and has proved a satisfactory mode of expression of the results. In 41 normal subjects the mean galactose time was 61 (range 30.92). The interval between the samples makes it impossible to record a galactose time less than 30 minutes. Further statistical treatment would therefore be of little use.

Intravenous hippuric acid synthesis test. The method employed was that of Quick *et al.* (1938) : 1·77 g. sodium benzoate in solution were injected intravenously and the subsequent hour's urine analysed for hippuric acid by the method of Weichselbaum and Probstein (1938-39). A urea clearance test was run in parallel with each test and always exceeded 80 per cent. of normal ;

BIOCHEMISTRY OF LIVER DISEASE
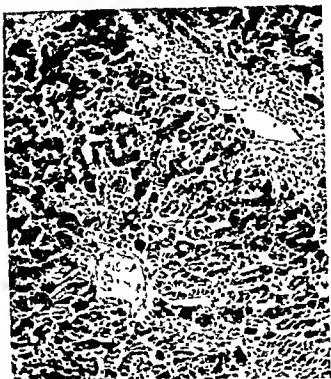
Fig. 1.—Grade A hepatitis. S., aged 46. Infectious hepatitis. Jaundiced 7 days. Serum bilirubin 5·6 mg., serum phosphatase 23 units, serum cholesterol 250 mg., total serum proteins 6 1 g., serum albumin 3·2 g., serum globulin 2·9 g., all per 100 ml. A/G ratio 1·1. Galactose time 08 mins. Hippuric acid excretion 0·85 g. Best's carmine stain. × 90.

Fig. 2.—Grade B hepatitis. L., aged 37. Arsenotherapy jaundice. 10 days jaundiced. Serum bilirubin 6 4 mg. Serum phosphatase 20 units, serum cholesterol 256 mg., total serum proteins 5·8 g., serum albumin 3·8 g., serum globulin 2 0 g., all per 100 ml. A/G ratio 1·9. Galactose time 60 minutes. Hippuric acid excretion 0 49 g. Best's carmine stain. × 90.
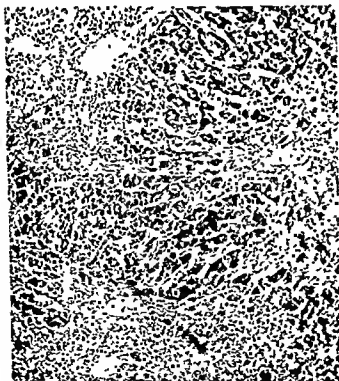
Fig. 3.—Grade C hepatitis. P. H., aged 20. Infectious hepatitis. Jaundiced 12 days. Serum bilirubin 0·9 mg., serum phosphatase 21 units, total serum proteins 4·5 g., serum albumin 2·4 g., serum globulin 2·1 g., all per 100 ml. A/G ratio 1·1. Galactose time 162 minutes. Hippuric acid excretion 0 36 g. Best's carmine stain. × 65.
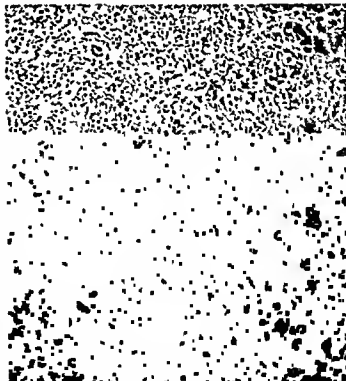
Fig. 4.—Grade D hepatitis. F. M., aged 60. Arsenotherapy jaundice. Jaundiced 9 days. Serum bilirubin 14·6 mg., serum phosphatase 17 units, serum cholesterol 217 mg., total serum proteins 6 8 g., serum albumin 2 8 g., serum globulin 4 0 g., all per 100 ml. A/G ratio 0 70. Galactose time 97 minutes. Hippuric acid excretion 0·49 g. Best's carmine stain. × 90.

there was no azotæmia. The urine volume during the test period was greater than 60 ml. The normal range for excretion was 1·21-0·75 g. hippuric acid expressed as sodium benzoate (Sherlock, 1946).

Mention of significant differences indicates that the data concerned have been subjected to statistical analysis.

CLINICAL MATERIAL

In all, 187 patients with diseases involving the liver have been studied. They have been simply grouped under four main headings.

(1) *Acute hepatitis* (84 cases). This group includes simple infectious hepatitis (21 cases), jaundice following the injection of icterogenic mumps convalescent serum or plasma infusions (9 cases), and jaundice occurring during the course of arsenical therapy for syphilis (54 cases). Apart from the incubation period and the history of arsenotherapy or injection of a presumably icterogenic serum, it is impossible to distinguish these three types clinically or histologically. The essential lesion is an acute inflammation with varying degrees of cell necrosis. According to the site of maximum change, zonal, diffuse or mixed diffuse and zonal types can be distinguished. Residual portal fibrotic lesions can also be recognised. These types blend into each other (Dible *et al.*, 1943). For the purpose of comparing the histological changes with the chemical findings it has been convenient to group the material according to the extent of liver cell damage, *i.e.* to the probable percentage of surviving hepatic cells (table I).

TABLE I

Histological grading of 84 cases of acute hepatitis

Grade	Probable percentage of surviving liver cells	No. of cases —
A	75-100	26
B	50-75	39
C	25-50	14
D	less than 25	5

The grading was done with numbered slides, the patients' names and the results of the various tests being at the time unknown. The grading was done by one person and the percentages quoted are only approximate. Examples are illustrated (figs. 1-4).

(2) *Cirrhosis of the liver* (26 cases). In accordance with modern terminology (de Josselin de Jong, 1931 ; Moon, 1932), cirrhosis of the liver has been applied to chronic diffuse liver disease with fibrosis, retrogressive parenchymal changes and regeneration of surviving cells (fig. 5). Instances of the latent type in which cell damage is minimal and the histological emphasis is on bands of mature fibrous tissue disrupting the normal architecture of the liver have been recognised (fig. 6). Recovered hepatitis with residual fibrous strands in the true anatomical portal tracts without disturbance of the essential hepatic architecture are not included (fig. 7).

(3) *Obstructive jaundice* (49 cases). In every instance there was complete obstruction to the common bile duct, confirmed at operation or autopsy. Pathological changes in the liver follow rapidly on occlusion of the common bile duct. The pertinent literature has been thoroughly reviewed by Cameron and Oakley (1932). In this series conspicuous liver changes were noted in every case. Bile pigment was present in the canaliculi, especially centrolobularly,

sometimes precipitated to form the so-called "bile thrombi". Focal bile-stained necroses, usually in relation to the periphery of the lobules, were also seen. There was an increase of fibrous tissue in the portal tracts, with pro-liferation of bile ducts. Disorganisation of liver architecture and massive damage to liver cells was a later feature. Some of these changes are illustrated (figs. 8 and 9).

4. *Miscellaneous diseases.* These included a wide variety of conditions in which there is liver involvement—hæmolytic jaundice (6 cases), hydatid cyst of liver (4 cases), kala azar (3 cases), involvement of liver in secondary neoplasms without production of jaundice (6 cases), amyloid disease of liver (4 cases), and single examples of Gaucher's disease, leuco-erythroblastic anæmia, histiocytic medullary reticulosis, amœbic abscess of liver and convalescent Weil's disease.

RESULTS

The results of the complete series are shown in table II.

(1) *Acute hepatitis*

The acute icteric stage

Serum bilirubin levels correlated well with the severity of the liver lesion. As damage to liver cells increases, so the mean serum bilirubin of the group rises. The wide range and large standard deviation in each group illustrate the variation from case to case. The statement, therefore, can only be a general one. It is, however, true that a serum bilirubin of more than 10 mg./100 ml. is usually associated with one of the severer grades of liver damage. Of 26 cases of histological grade "A" only one had a higher level. The reverse, namely, that a low bilirubin is associated with mild liver damage, is usually but not invariably true.

Serum phosphatase. In all grades there was a moderate rise in serum phosphatase, but in only 4 of the 84 patients with acute hepatitis did the level exceed 30 units/100 ml. (fig. 10). In contrast to the serum bilirubin, there was no significant difference between the means for the various grades of histological severity. Consequently a positive correlation between serum bilirubin and phosphatase could not be established.

Serum cholesterol in 39 patients was within the normal range. In 14 others the level was above the upper normal limit of 230 mg./100 ml. There was no significant difference between the mean serum cholesterol for the various histological grades.

Serum proteins. The mean total serum protein for 41 cases was 6·04, that is, about the lower limit of normal. The serum albumin, serum globulin and the A/G ratio also fell within the normal range. Comparison of the serum proteins in the various histological grades of hepatitis shows a significant difference between the less severe (A and B) and the more severe (C and D) grades. In the more severe grades the total serum protein level was lower and the serum albumin more reduced. The cases in the group of maximal severity (D) showed

PLATE LXXXVII

BIOCHEMISTRY OF LIVER DISEASE
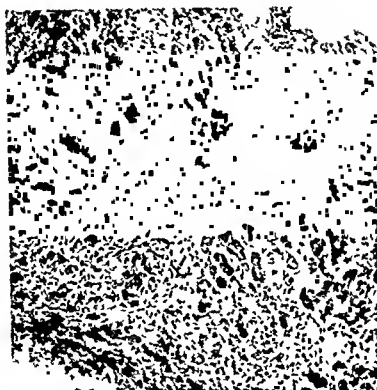
FIG. 5 —Active cirrhosis. M. T., aged 40. Serum bilirubin 9·2 mg., serum phosphatase 19 units, serum cholesterol 212 mg., total serum proteins 6·7 g., serum albumin 2·6 mg., serum globulin 4·1 g., all per 100 ml. A/G ratio 0·64. Galactose time 108 minutes. Hippuric acid excretion 0·4 g. Best's carmine stain. × 100.
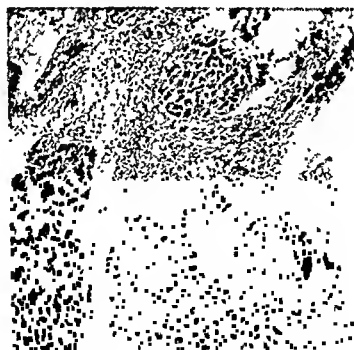
FIG. 6 —Latent inactive cirrhosis. A. C., aged 32. Serum bilirubin 0·5 mg., serum phosphatase 4 units, total serum proteins 6·8 g., serum albumin 4·8 g., serum globulin 2·0 g., all per 100 ml. A/G ratio 2·4. Galactose time 33 minutes. Hippuric acid excretion 1·05 g. Best's carmine stain. × 65.

significant hyperglobulinæmia and a reversal of the A/G ratio. The only 3 fatal cases were in this group, and all had serum albumin levels of 3 g./100 ml. or less. Apart from one case, a very undernourished old woman, levels of serum albumin of 3 g. or less were encountered only in the severe histological grades of hepatitis.

Galactose time. In 15 of 49 patients examined, galactose tolerance
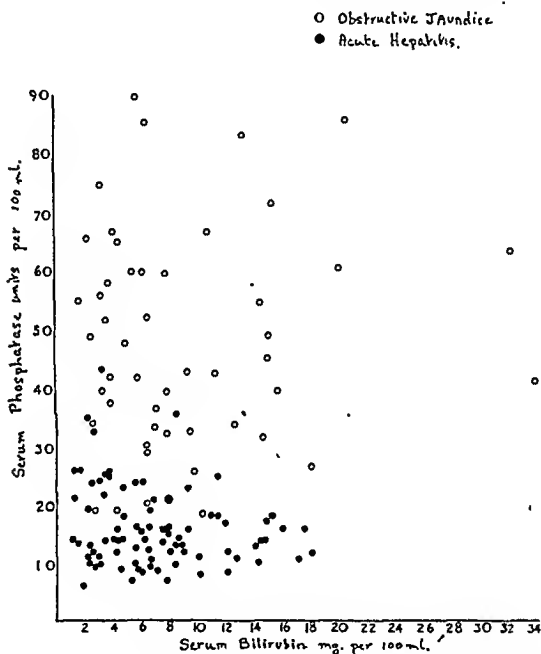
FIG. 10.—Serum bilirubin and phosphatase in acute hepatitis and obstructive jaundice.

was better than the mean value for normal subjects. Subdivision of cases of hepatitis into varying degrees of histological severity shows that the cases with normal tolerance usually fall into the grade with the least degree of liver cell damage. There is a progressive increase of galactose time with increasing histological severity of hepatitis.

Hippuric acid synthesis. In 10 of 40 patients examined, the results of the test were within normal limits. A close correlation could not be established between the severity of the hepatitis and the

TABLE II

Biochemical results in 187 cases of liver disease

| | Serum bilirubin (mg./100 ml.) | | Serum phosphatase (units/100 ml.) | | Serum cholesterol (mg./100 ml.) | | Serum proteins (g./100 ml.) | | | | | Galactose time (minutes) | | Hippuric acid excretion (g.) | |
| | | | | | | | Total | | Albumin | Globulin | | | | | |
	No. of cases	Mean	No. of cases	Mean	No. of cases	Mean	No. of cases	Mean	Mean	Mean	No. of cases	Mean	No. of cases	Mean
Normal	...	(1·5-0·5)	...	(13-4)	...	(230-120)	...	(8-6)	(5-3·4)	(3-1·5)	...	61 (90-30)	...	(1·2-0·75)
Acute hepatitis	84	6·77 / 0·45* / (18-1·1)†	84	15·6 / 0·77 / (43-7)	53	193 / 6 / (285-110)	41	6·0 / 0·7 / (8·1-4·5)	3·5 / 0·12 / (4·7-2·0)	2·54 / 0·13 / (4-7·1)	49	82 (184-30)	40	0·63 / 0·055 / (1·32-0·18)
Grade A	25	3·4 / 0·62 / (12·8-1·1)	26	15·1 / 1·07 / (25-7)	19	198 / 11·6 / (285-111)	12	6·4 / 0·4 / (8·1-5·2)	3·7 / 0·03 / (4·6-3·1)	2·7 / 0·2 / (4-7·1)	15	55 (68-30)	13	0·9 / 0·055 / (1·3-0·72)
Grade B	39	7·0 / 0·4 / (17·4-2·2)	39	17·4 / 0·63 / (43-7)	22	194 / 10·4 / (266-124)	19	6·1 / 0·15 / (7·1-4·8)	3·8 / 0·14 / (4·7-2·1)	2·3 / 0·2 / (3-6·1)	24	86 (127-35)	19	0·5 / 0·04 / (0·91-0·18)
Grade C	14	9·7 / 0·88 / (18-5·3)	14	17 / 1·78 / (33-8)	8	195 / 10·6 / (250-110)	6	5·6 / 0·21 / (6·2-4·5)	3·0 / 0·27 / (3·8-2·4)	2·6 / 0·05 / (3·0-2·1)	8	113 (184-30)	7	0·5 / 0·53 / (0·84-0·36)
Grade D	5	14·4 / 0·98 / (17-9·8)	5	13 / 0·84 / (17-10)	4	175 / 7·5 / (217-110)	4	5·8 / 0·3 / (6·8-5·0)	2·6 / 0·14 / (3·0-2·0)	3·2 / 0·45 / (4·0-2·0)	2	101 (104-97)	1	0·5
Hepatic cirrhosis active	17	5·0 / 0·85 / (11·2-1·2)	16	16 / 1·9 / (36-7)	15	202 / 13 / (327-120)	12	6·8 / 0·36 / (9·0-4·0)	2·8 / 0·09 / (3·7-2·0)	4·0 / 0·3 / (5·5-2·0)	10	106 (134-69)	10	0·41 / 0·3 / (0·6-0·2)

Latent	9	0·73 0·08 (1 0·0·5)	9	12·0 2·7 (25·4)	8	182 12 (208-140)	8	6·7 0·42 (7·2-5·0)	4·2 0·25 (4·8-3·5)	2·5 0·14 (3·3-2·0)	7	52 (72·4)	8	0·87 0·05 (1·05-0·0)
Obstructive jaundice	49	0·1 0·99 (34·2 0)	49	48·6 2·6 (90·18)	20	202 10 (550-167)	12	0·0 0·28 (8·3-3·0)	3·3 0·18 (4·7-1·0)	2·73 0·11 (3·0-2·0)	18	89 (158-30)	23	0·5 0·08 (1·03-0·10)
Hæmolytic jaundice	0	3·4 0·24 (3·1)	0	5·7 0·14 (7·4)	4	182 (225-131)	5	0·4 (7·9-5·4)	4·2 (4·9-3·3)	2·9 (3·2-2·1)	4	45 (51·35)	5	0·5 (0·10-0·2)
Miscellaneous blood diseases	3	0·5	3	7·3	3	184	3	6·5	3·8	2·7	1	63	1	0·7
Hydatid cyst	4	0·5	4	10·6 (10·3-3)	4	255 (302-118)	4	0·3 (0·8-5·8)	3·7 (3·8-3·0)	2·0 (3·0-2·2)	2	07	3	0·00 (1·3-0·73)
Amœbic abscess	1	5·1	1	31	1	...	1	0·0	3·0	2·1	1	30	1	0·05
Weil's disease	1	2·3	1	10	1	260	1	4·8	2·4	2·4	1	30	...	...
Kala azar	3	0·5	3	12 (18·0)	3	183 (189-177)	3	0·0 (7·0-0·0)	4·4 (4·0-1·1)	2·5 (2·9-2·0)	3	31	3	1·00 (1·21-0·97)
Amyloid disease	4	0·5	4	14 (21-10)	4	214 (230-173)	2	5·8	2·8	3·0	2	05	2	0·91
Secondary malignant disease	6	0·71 0·15 (1·5-0·5)	5	11·0 (23·6)	4	200 (216-147)	6	5·5 (0·1-5·1)	3·5 (4·4-2·4)	2·0 (3·2-1·0)	3	50	5	0·5 (1·0-0·3)

* = standard error of mean. † = range.

excretion of hippuric acid. In grade A the mean hippuric acid excretion falls within normal limits (0·9 g.). · The severer grades (B, C and D) show much lower means. These grades, however, in spite of the increasing extent of liver cell necrosis, do not show decreasing hippuric acid excretion. In fact, there is no significant difference between the mean values in grades B, C and D. A basic low level (0·45-0·6) exists in the severer forms of hepatitis, and excretion does not often fall below this, however severe the liver damage.

Changes during recovery from acute hepatitis

Serum bilirubin. Patients were rarely admitted to hospital during the pre-icteric phase; the disease, moreover, is usually of limited duration, and the trend of the bilirubin readings is downward. The levels recorded during the first week in hospital are usually the highest. In only 3 out of 30 cases did serial serum bilirubins show an increase of jaundice after this period. In these 30 patients the serum bilirubin was followed at intervals of not more than seven days until a level below 2 mg./100 ml. was reached. The bilirubin of all the cases of minimum histological severity (grade A) fell to below 2 mg. in less than 3 weeks. The more severe cases took longer. There was a correlation between the time required and the initial histological severity of the hepatitis. Similarly, the cases with the highest initial serum bilirubin took the longest time to reach 2 mg./100 ml. (fig. 11). The correlation coefficient between the maximum bilirubin and the duration of bilirubinæmia is 0·7 for 30 cases. This is statistically significant. The bilirubin level does not show a constant rate of fall as recovery proceeds. The initial fall is very rapid, especially from the higher figures; the eventual fall to normal is slower. That the serum bilirubin may remain slightly elevated (1-2 mg./100 ml.) for a week or two after apparent clinical recovery does not imply the development of a subacute or chronic hepatic lesion. The liver histology is nearly always normal in these instances and the excess bilirubinæmia soon disappears.

Serum phosphatase did not show the steady fall characteristic of serum bilirubin. In 19 of 30 patients with acute hepatitis, the serum phosphatase fluctuated during the recovery period and a longer time was required to reach a normal level than that needed for the serum bilirubin. When the serum bilirubin had reached 2 mg./100 ml., in only 10 out of 30 cases was serum phosphatase as low as 10 units. On discharge from hospital 12 cases still showed a raised serum phosphatase. In the 7 cases in which circumstances permitted observation of the level over the succeeding twelve months, all eventually returned to normal values. In general, the patients in whom initial histological damage to the liver was greatest took the longest time for the serum phosphatase to reach normal. There were, however, exceptions to this rule. The slowly falling phosphatase

BIOCHEMISTRY OF LIVER DISEASE
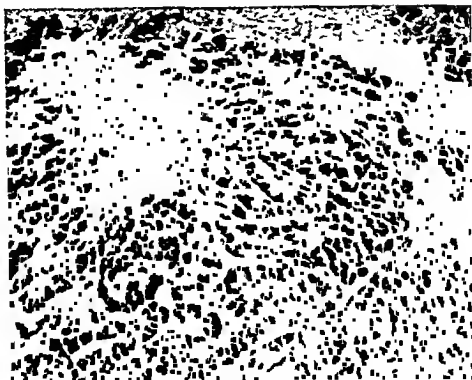
Fig. 7.—Residual portal scarring following hepatitis. Same case as in fig. 4, 33 days after onset of jaundice. Serum bilirubin 1·5 mg., serum phosphatase 12 units, total serum proteins 6·2 g., serum albumin 3·6 g., serum globulin 2·6 g., all per 100 ml. A/G ratio 1·4. Galactose time 46 minutes. Hippuric acid excretion 0·72 g. Best's carmine stain. ×100.
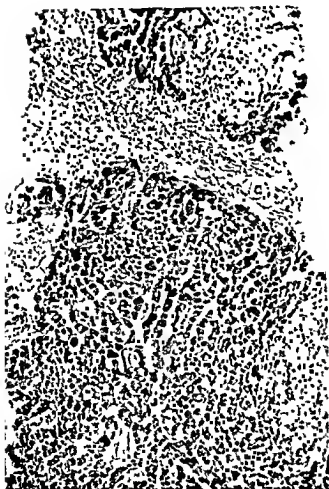
Fig. 8.—Obstructive jaundice. Bile-stained necroses at periphery of lobule. Increased fibrous tissue and bile duct proliferation in a portal tract. O. D., aged 47. Jaundiced 37 days. Serum bilirubin 15 mg., serum phosphatase 49 units, serum cholesterol 372 mg., all per 100 ml. Galactose time 47 minutes. Hippuric acid excretion 0·2 g. Best's carmine stain. ×85.
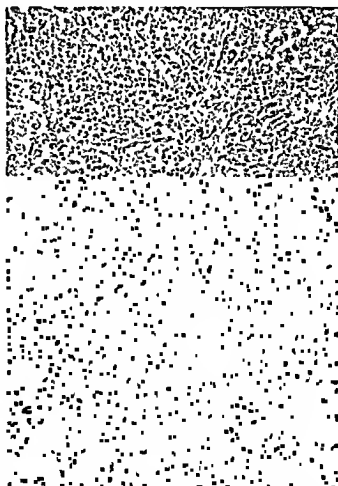
Fig. 9.—Obstructive jaundice. Early biliary cirrhosis. W. R., aged 37. Jaundiced 100 days. Serum bilirubin 12·5 mg., serum phosphatase 33 units, serum cholesterol 256 mg., total serum protein 6·4 g., serum albumin 3·1 g., serum globulin 3·3 g., all per 100 ml. A/G ratio 0·94. Galactose time 102 minutes. Hippuric acid excretion 0·4 g. H and E. ×60.

was not reflected in the progress of the histological recovery. In 14 patients in whom the phosphatase varied during recovery a follow-up biopsy was performed during the recovery phase and the usual progress of rapid cellular restoration was confirmed.

Serum cholesterol sometimes showed a rise during recovery, but this was inconstant.
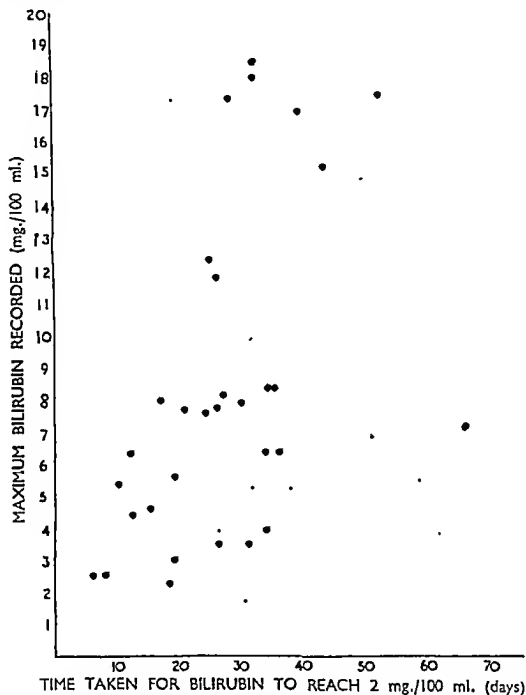
FIG. 11.—Acute hepatitis: duration of bilirubinæmia (in days) contrasted with the maximum serum bilirubin recorded.

Serum proteins. Although, as mentioned above, the serum proteins in the acute stage were within normal limits, the actual divergence from normal for that particular group is shown when the values after recovery are estimated. This comparison was done in 21 cases (table III). On recovery, the total serum protein did not alter significantly; there was, however, a rise in serum albumin, a fall in serum globulin and a rise in the A/G ratio. These changes are

TABLE III

*The serum proteins before and after recovery
in 21 cases of acute hepatitis*

	Mean duration of jaundice (days)	Total serum protein (g./100 ml.)		Serum albumin (g./100 ml.)		Serum globulin (g./100 ml.)		A/G ratio	
		Mean	S.E. of mean	Mean	S.E. of mean	Mean	S.E. of mean	Mean	S.E. of mean
Acute stage .	12	6·02	0·17	3·41	0·14	2·6	0·11	1·31	0·11
After recovery	32	6·51	0·14	4·25	0·15	2·25	0·12	1·9	0·16

statistically significant though occurring within the wide normal range of values.

Galactose tolerance is impaired more often in the first 14 days than later in the disease. The earlier the case is studied, the more likely is the galactose tolerance to be abnormal (fig. 12). In 13 cases in
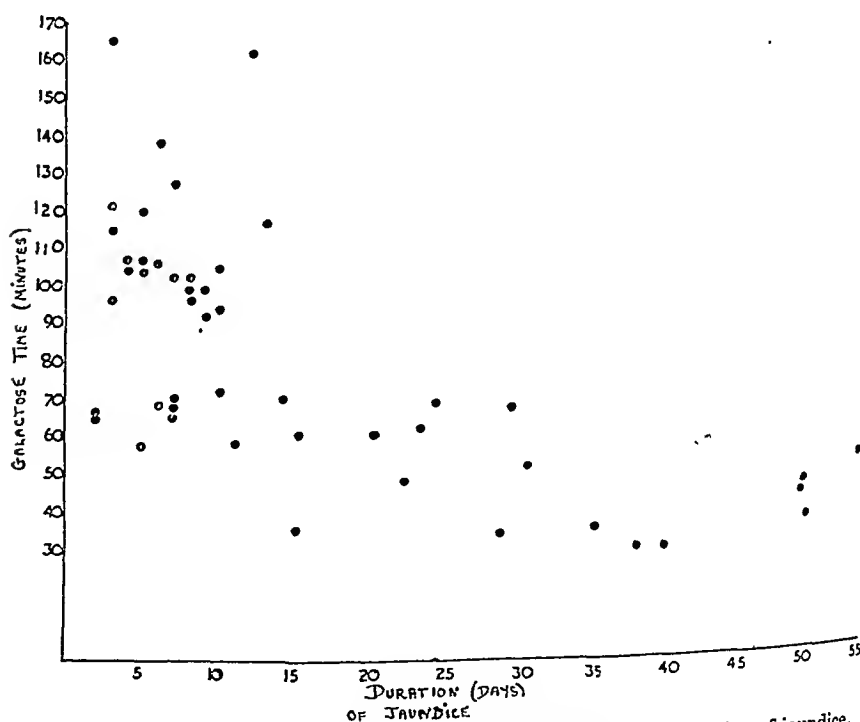
FIG. 12.—Acute hepatitis : relation between galactose time and duration of jaundice.

which it was initially abnormal the test was repeated later, with the results seen in table IV. All had returned to normal in an average of 13 days after the first test. In 10 cases, despite the normal galactose tolerance, the serum bilirubin was still greater than 2 mg./100 ml. In 9 of the cases a second liver biopsy was done at the time of the

TABLE IV

Acute hepatitis: time taken for restoration of normal galactose tolerance

Case	Sex	Age	Histological grade	Impaired galactose tolerance			Normal galactose tolerance		
				Duration of jaundice (days)	Serum bilirubin (mg./100 ml.)	Galactose time (minutes)	Duration of jaundice (days)	Serum bilirubin (mg /100 ml.)	Galactose time (minutes)
1	M	37	B	3	4·4	06	18	2·1	45
2	M	27	B	8	8·4	07	28	17·0	71
3	M	21	B	7	3·5	102	23	2·5	68
4	M	58	B	4	2·5	104	16	0·5	70
5	M	41	B	0	8·0	106	11	2·6	76
6	M	34	B	7	5·4	127	10	1·2	03
7	M	34	B	14	3·4	117	31	1·1	65
8	M	41	B	0	11·2	92	17	3·8	40
9	M	28	C	10	11·5	04	18	6·0	53
10	M	38	C	10	18·0	105	17	3·3	73
11	F	20	C	12	7·1	162	24	0·5	01
12	M	45	C	3	8·4	151	30	2·6	63
13	M	01	D	7	18·0	67	18	14·0	67
Mean				8	8·4	112	21	4·0	63

second tolerance test. In 4 the recovery of the power to metabolise galactose coincided with complete histological recovery. In the other 5, all cases of great initial histological severity, evidence of acute hepatitis remained. In 2 of these 5 cases a third (later) biopsy demonstrated complete histological recovery.

TABLE V

Acute hepatitis: time taken for restoration of a normal intravenous hippuric acid test

Case	Sex	Age	Histological grade	Impaired hippuric acid test			Normal hippuric acid test		
				Duration of jaundice (days)	Serum bilirubin (mg./100 ml.)	Hippuric acid (g)	Duration of jaundice (days)	Serum bilirubin (mg /100 ml)	Hippuric acid (g)
1	F	20	A	0	3·1	0·65	10	1·6	0·87
2	F	24	A	28	2·2	0·72	44	1·0	0·93
3	M	46	A	7	5·6	0·85	20	1·3	0·95
4	M	41	B	8	8·0	0·21	23	1·2	1·01
5	M	21	B	7	3·5	0·71	41	2·5	0·63
6	M	41	B	14	12·0	0·3	33	2·3	1·1
7	M	18	B	4	3·0	0·8	34	1·3	1·2
8	M	37	B	15	0·4	0·49	45	1·2	0·86
9	M	33	B	10	6·2	0·2	61	2·6	0·90
10	M	27	B	8	8·4	0·18	17	1·4	1·04
11	M	38	C	10	18·0	0·38	31	3·3	0·84
12	M	28	C	0	11·5	0·63	25	1·0	0·84
13	M	41	C	12	12·0	0·40	50	1·5	0·83
14	F	20	C	7	7·1	0·30	03	2·0	1·08
15	M	01	D		13·8	0·49		0·5	0·86
Mean				10	8·1	0·49	35	1·7	0·05

Hippuric acid synthesis. In 24 patients the hippuric acid excretion test, initially abnormal, was repeated after clinical recovery. In 19 excretion had returned to normal. In the other 5 the test was normal when a follow-up examination was made 3 months after discharge from hospital. In 15 cases, tests were performed at weekly intervals during the course of the illness (table V). The mean time taken for the hippuric acid excretion to rise from a low to a normal value was 25 days (range 17-63). The patients whose livers showed the greatest initial histological severity took the longest time to regain the normal power of synthesis. Return of normal hippuric acid synthesis, however, often followed 2-5 weeks after the liver had recovered its
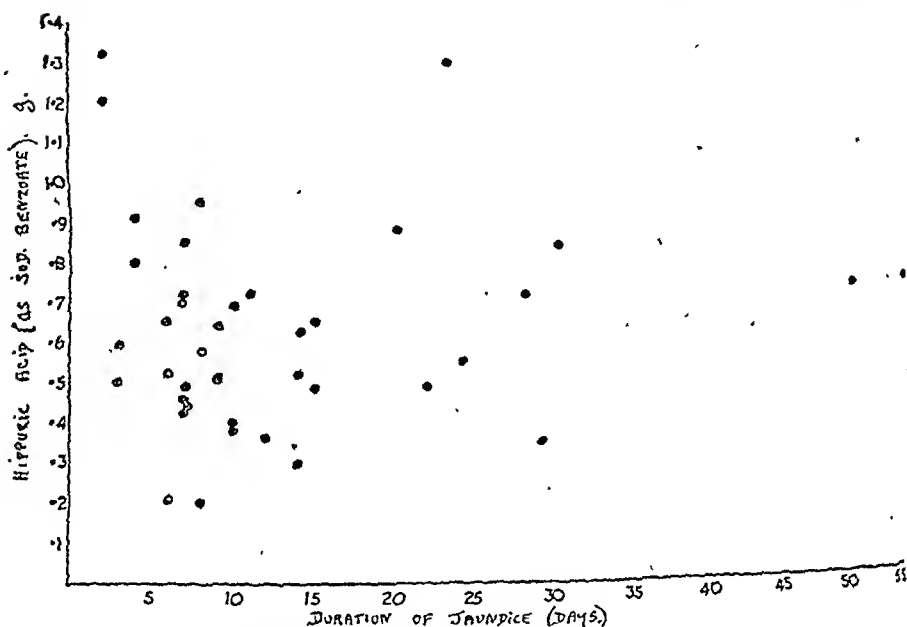
FIG. 13.—Acute hepatitis : relation between hippuric acid test and duration of jaundice.

normal histological appearance. There appeared to be no correlation between the excretion of hippuric acid and the period of jaundice at which the test was performed (fig. 13).

(2) *Hepatic cirrhosis*

Histologically inactive cirrhosis

Serum bilirubin in all the 9 cases was less than 1·0 mg./100 ml.

Serum phosphatase. Four of the 9 patients showed a serum phosphatase of greater than 10 units/100 ml. In 2 this was the only abnormality detected.

Serum cholesterol. One patient showed a raised level. The others were within normal limits.

Serum proteins were estimated in 8 patients : 7 were normal; in the eighth the globulin was 3·3 and the A/G ratio 1·1.

Galactose tolerance was estimated in 8 patients. The sugar was eliminated from the blood during the test period. The highest galactose time was 72 minutes.

Hippuric acid synthesis was normal in 6 of 8 patients. The other 2 showed values only slightly below normal limits.

Histologically active cirrhosis

Serum bilirubin was moderately raised in all 17 patients. The highest level recorded was 11·2 mg./100 ml. In 12 of the 17 patients the disease proved fatal during their stay in hospital. The height of the serum bilirubin in these cases did not serve as a means of predicting the fatal outcome, nor was it correlated with the extent of liver damage as seen by biopsy or at autopsy. Where multiple readings were available, fluctuations of about 3 mg./100 ml. were usually observed, and these did not correlate with changes in the histological appearance of the liver or in the clinical course.

Serum phosphatase was very variable : 13 of 16 patients showed a level above 10 units/100 ml., but only 1 was greater than 30 units/100 ml. There was no correlation between the degree of hepatic cell damage and the level of the phosphatase.

Serum cholesterol was normal in 11 of the 15 patients in whom it was estimated. In 4 there was a raised figure, and in 2 there was associated chronic alcoholism. The serum cholesterol could not be compared with the extent of the hepatic lesion.

Serum proteins. Of the 12 patients studied, all except 1 showed a normal total serum protein level. (This was a man who had had a severe haematemesis 48 hours previously and who showed a very low level). In the other cases the components of the serum protein had been greatly altered. Of the 11 cases, 9 had a serum albumin of less than 3·4 g /100 ml. and in 7 of them the level was below 3 g. The disease proved quickly fatal in these 7 cases. The serum globulin in 10 of the 11 was greater than 3 g./100 ml. and in 8 the value was greater than 4 g. There was a resulting change in the A/G ratio. In all 12 patients the ratio was less than 1·3, and in 10 the ratio was reversed.

Galactose tolerance. Nine of 10 patients gave conspicuously abnormal results for the intravenous galactose tolerance test. The degree of impairment was in proportion to the extent of cell necrosis and in inverse ratio to the extent of cell regeneration.

Hippuric acid synthesis was impaired in the 10 cases in which it was tested. The amount excreted did not correlate with the apparent extent of liver damage.

(3) Obstructive jaundice

Serum bilirubin. For purposes of comparison the serum bilirubin in patients with complicating gross biliary cirrhosis or widespread

hepatic metastases has been compared with that found in cases in which the obstruction to the common bile duct was associated with minimal liver changes. Both groups were of approximately the same duration. The mean bilirubin in each group showed no significant difference. In 6 cases of unrelieved obstruction the bilirubin rose steadily for the first three weeks of jaundice; the level then fluctuated, the trend being upward.

Serum phosphatase was raised. In only 7 of the 49 cases did the level fall below 30 units/100 ml. (fig. 10). In the course of obstructive jaundice the level usually rises progressively. Table II (pp. 528 and 529) is constructed from the first serum phosphatase of each patient recorded on admission to hospital. In 17 patients further readings were available during the course of the illness; the mean rose from 52 to 80 units/100 ml. (mean of highest values reached).

The high serum phosphatase is present from a very early stage and does not reflect the secondary changes occurring in the liver. Progressive liver damage in obstructive jaundice as revealed by serial hepatic biopsies does not influence the upward trend of the serum phosphatase during the course of the illness. The level is not significantly different where massive hepatic metastases are associated with the obstruction. Extreme cachexia does not influence the very high levels reached in the last stages of obstructive jaundice due to carcinoma. A positive correlation could not be established between serum bilirubin and serum phosphatase.

Serum cholesterol was usually raised, but there was much individual variation (table II): 18 of 39 cases had levels less than 260 mg./100 ml. The serum cholesterol did not fall with increasing duration of obstruction. The level did not reflect the grade of associated hepatic changes. A statistically significant correlation could not be established between serum·cholesterol and·either serum phosphatase or serum bilirubin.

Serum protein. In 12 patients the mean total serum protein was low. This was attributed to the fall in albumin, the globulin being almost unaltered. Of the 12 cases 7 had a serum albumin of 3 g./100 ml. or less. The total serum protein and the serum albumin diminished with increasing duration of obstruction and cachexia.

Galactose tolerance was frequently impaired. In 15 of 18 cases of obstructive jaundice the galactose time was greater than the mean normal time. Galactose tolerance becomes more impaired as the duration of obstruction increases. No definite correlation could be established between the galactose time and the duration of icterus. However, 5 cases of obstruction which were not surgically relieved, and which showed initially a normal " galactose time ", were followed through the course of the illness. The galactose tolerance of all eventually became impaired. There is little relation between the level of the serum bilirubin and the galactose time.

Hippuric acid synthesis was usually impaired. Of 22 patients

examined, 20 showed excretion below the lower limit of normal. Lower values were not recorded in those with advanced hepatic changes. The lowest hippuric acid excretion was found in patients obstructed longest and with the deepest icterus.

(4) *Miscellaneous diseases involving the liver*

The biochemical methods studied usually gave normal results. Exceptions were a raised *serum bilirubin* in a case of amœbic abscess, a case of Weil's disease, and one of the six cases of hepatic secondary neoplasm. *Serum phosphatase* also was sometimes raised and values above normal were encountered in amœbic abscess, Weil's disease and secondary malignant disease. The level was usually less than 30 units/100 ml. The *hippuric acid synthesis* test gave variable results and could not be related to liver histology.

DISCUSSION

Clinicians have long been dubious of the practical value of most of the laboratory aids used in the study of liver diseases. This investigation of some of the more commonly used methods, correlated with the histological appearance of the liver, supports this contention. Soffer (1935), in his comprehensive review of liver function tests, states that normal values do not exclude the presence of hepatic disease. This is borne out by the present series. All types of liver disease—acute hepatitis, cirrhosis, both primary and that secondary to biliary tract disease, massive malignant metastases and cysts—have been associated with normal results for all the tests studied. The converse is not common. Tables VI and VII present a summarised

TABLE VI

The practical differential diagnostic value of the laboratory methods described

Diagnosis	Serum bilirubin (mg /100 ml)	Serum phosphatase (units/100 ml)	Serum cholesterol (mg /100 ml)	Serum proteins (g /100 ml)		Galactose time (minutes)	Intravenous hippuric acid test (g)
				Total	Albumin		
Acute hepatitis	Useless	Usually less than 30	Usually less than 300	Useless	Useless	Useless	Useless
Cirrhosis active	Usually less than 12	,,	,,	,,	,,	Usually abnormal	Usually abnormal
Latent	Usually less than 1	,,	,,	,,	,,	Usually normal	Usually normal
Obstructive jaundice	Useless	Usually greater than 30	Useless	,,	,,	Useless	Useless

TABLE VII

The practical value of the laboratory methods described in assessing the extent of the surviving liver tissue

Diagnosis	Serum bilirubin (mg./100 ml.)	Serum phosphatase (units/100 ml.)	Serum cholesterol (mg./100 ml.)	Serum proteins (g./100 ml.)		Galactose time (minutes)	Intravenous hippuric acid test (g.)
				Total	Albumin		
Acute hepatitis	High values associated severe lesions	Useless	Useless	Useless	Usually less than 3 in very severe lesions	High values in severe lesions	Useless
Active cirrhosis	Useless	Useless	Useless	Useless	Usually less than 3 in severe cases	Correlates well with severity of lesion	Useless

evaluation of the methods of investigation employed. Sections of the liver in acute hepatitis have proved most useful for grading the extent of parenchymal involvement. Fig. 14 shows the effect of the increasing hepatic damage on the mean results of the biochemical investigations. It has been compiled from table II.

The bilirubinæmia of acute hepatitis is attributed to an intralobular obstruction to the excretion of bile. The liver cell columns with their intercellular bile canaliculi are disrupted and the surviving liver cells may be quantitatively inadequate to excrete the bile pigment brought to them in the blood stream (Dible *et al.*, 1943). It is not surprising, therefore, that in the acute phase of hepatitis the height of the serum bilirubin correlates well with the extent of the hepatic cell destruction. During this phase the reticular framework of the liver has usually remained intact ; with recovery the liver cells fall into regular columns and there is coincident restitution of the bile canaliculi. The speed of histological recovery is such that the initial fall of serum bilirubin is very rapid (*cf.* figs. 4 and 7). The occurrence of a slightly raised serum bilirubin after complete histological and clinical recovery may be due to slow excretion of bilirubin, which may have been bound to tissue protein. The persistence of skin and conjunctival staining after clinical recovery is well known. It may also be influenced by a rise during recovery of the renal threshold for bilirubin (Gellis and Stokes, 1945).

The serum phosphatase did not diminish with increasing severity of hepatitis. The liver, therefore, is not the main source of the increase in serum phosphatase found in jaundice. The suggestion of Armstrong and King (1935) that the level might be proportional to the degree of liver damage and of Drill and Ivy (1944) that the phosphatase increase might be used to detect liver damage has not been substantiated. The cause of the serum phosphatase fluctuations during recovery is not known.

The serum cholesterol is affected by circumstances not directly related to the liver, such as fever, cachexia and starvation (Jones, 1942). Moreover, the wide normal range of serum cholesterol makes interpretation of small changes difficult. The measurement of the proportion of esterised to free cholesterol—a more constant figure in normal subjects—might have provided more useful information, but
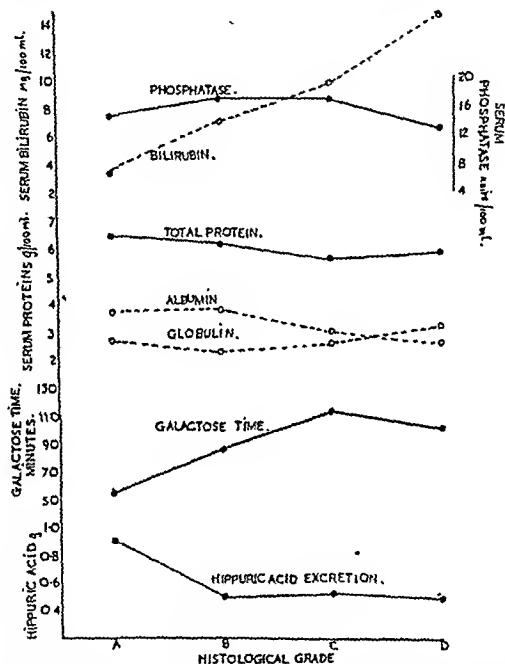
Fig. 14.—Acute hepatitis: biochemical changes with increasing hepatic cell damage.

owing to pressure of work on the reduced war-time staff, this was not added to the number of tests already in use. In acute hepatitis the fall of cholesterol in the severe case suggested by Epstein (1937) was not observed. There was no variation between the mean of the different grades of severity. The estimation gave no useful diagnostic or prognostic information.

In acute hepatitis the total serum protein usually falls within the normal range. Serum albumin is reduced in the more severe degrees of histological damage, a fact in keeping with the known hepatic source of the substance. Globulin also may have an extra-hepatic

origin (Sabin, 1939), and is not reduced to the same extent. A rise in globulin (United States Army Instruction, 1944) was rarely encountered.

The failure of the galactose tolerance test to detect lesser degrees of liver damage is well known (Soffer, 1935; Krarup, 1943; Drill and Ivy, 1944). The progressive increase of galactose time with increasing histological severity of hepatitis suggests that galactose tolerance is a true measure of liver function; acute diffuse liver damage is, however, essential before tolerance is impaired. This may be explained by the histological picture of acute hepatitis. The surviving liver cells are seen to have a normal complement of glycogen and may retain their power of metabolising glycogen. It is not surprising, therefore, that tests of the carbohydrate functions of the liver run parallel with the percentage of surviving liver cells. Moreover, because of the great reserve power of the liver, the cases of minimal histological severity will show little or no impairment of galactose tolerance. Liver damage in hepatitis is maximal early in the disease; therefore an impaired galactose tolerance test is encountered most often in the first 14 days of illness. The method is insensitive; therefore galactose tolerance returns to normal before histological recovery is complete.

The normal hippuric acid excretion in grade A hepatitis is associated with the minimal hepatic cell damage present, the main histological change being cellular accumulation in the portal tracts. The failure of the mean hippuric acid excretion to decrease with increasing liver cell necrosis is unexpected. Moreover, in contrast to results of the galactose tolerance test, hippuric acid synthesis does not return to normal with complete structural recovery. These facts suggest that the changes in hippuric acid synthesis in acute hepatitis are not dependent only on the liver.

Study of the findings in the series of acute hepatitis cases has failed to reveal any biochemical distinction between "simple" infectious hepatitis and hepatitis following arsenotherapy or the parenteral administration of icterogenic sera. The arsenotherapy cases have usually fallen into the severer histological grades.

Hepatic cirrhosis could not be detected in the absence of acute liver cell destruction. It is in the inactive type, where the clinical features are equivocal, that accurate diagnosis is essential. Active cirrhosis usually presents an obvious clinical picture and the biochemical abnormalities so constantly demonstrated are merely confirmatory.

The bilirubinæmia of cirrhosis is dependent upon the balance between liver cell necrosis and regeneration. A raised serum bilirubin in cirrhosis indicates inadequately compensated destruction of liver cells or disruption of the normal lobular architecture and bile duct system, producing an intralobular obstruction to the excretion of bile. The great regenerative capacity of the liver explains why the serum

bilirubin in cirrhosis rarely rises above 12 mg./100 ml. The mechanism of the moderately raised serum phosphatase in acute cirrhosis cannot be explained in the present state of knowledge.

Alterations in the serum proteins in cirrhosis are well known (Grenot, 1907 ; Gilbert and Chiray, 1907 ; Tumen and Bockus, 1937). The changes show a similar pattern to those described for acute hepatitis. Total protein is normal, serum albumin low and serum globulin raised. The changes in albumin and globulin are much more conspicuous than those seen in hepatitis, perhaps because of the chronicity of the condition. Post and Patek (1942) believe that serum albumin levels are of prognostic use. This has been confirmed, and serum albumin levels correlate well with the extent of liver necrosis as seen in sections.

Galactose tolerance was usually impaired in active cirrhosis and the extent of impairment could be associated with the severity of the underlying hepatic lesion. The test is time consuming and the results obtained give little information not obtained by the protein estimations. Hippuric acid synthesis also usually showed impairment but, as in acute hepatitis, the results could not be correlated with the histological picture.

Serum bilirubin, cholesterol and proteins and the galactose and hippuric acid tests were of little use in the diagnosis of obstructive jaundice from acute hepatitis and cirrhosis with jaundice. The raised serum phosphatase of obstructive jaundice (Roberts, 1930 ; Herbert, 1935) has proved of practical diagnostic value. If an arbitrary level of 30 units/100 ml. is taken, most cases of hepatitis and cirrhosis show levels less than this, and most cases of obstructive jaundice fall above it. The occasional exception is a patient with obstructive jaundice whose serum phosphatase is less than 30. It is stated that serum cholesterol is raised in obstructive jaundice (Flint, 1862 ; Epstein and Greenspan, 1936), but in the present series this proved a much less constant finding than the increase in phosphatase. However, a serum cholesterol level of more than 300 mg./100 ml. is unusual in jaundice other than that due to obstruction of the common bile duct.

The serum protein changes in obstructive jaundice did not show the pattern described for primary parenchymatous jaundice. Total serum protein and serum albumin were low, but the globulin showed no increase. The changes are probably due more to the poor general condition of the patients than to the secondary changes in the liver. This probably applies also to the hippuric acid synthesis test (Sherlock, 1946), the results of which did not correlate well with the extent of the hepatic lesions. Although the high incidence of abnormal galactose tolerance in obstructive jaundice prevented the diagnostic use of the method, there was good correlation between the length of the galactose time and the extent of the underlying hepatic lesion. Galactose tolerance alone of the methods used reflected these secondary changes

and can be used as a pre-operative index of the extent of hepatic involvement in obstructive jaundice.

In the miscellaneous group results were usually within normal limits. This is not surprising, as the liver is involved either focally or by abnormal portal tract and sinusoidal cell accumulations. The reserve power of the liver is not impaired.

The more laboratory investigations are multiplied, the greater likelihood is there of a biochemical deficiency being demonstrated. To the clinician, anxious to make a diagnosis and to assess prognosis, this type of "shotgun" investigation may well add to the confusion rather than give a clear-cut answer. Many of the theories of liver function are based on the results of animal experiment. In man, conditions are much more complex. Dehydration, cachexia, fever and renal disease are frequent accompaniments of human hepatic disease. An uncomplicated hepatic lesion is rare. Of the estimations studied, only the serum phosphatase and the intravenous galactose tolerance test seem free from changes brought about by these non-hepatic factors. Before final judgment can be passed on the usefulness of these laboratory aids, much more information is needed of their exact relation to hepatic damage in man. In the meantime, in the jaundiced subject, a single venous blood sample which is analysed for serum bilirubin and alkaline phosphatase gives as much practical diagnostic information as the more complicated procedures. If facilities are available, the differential serum proteins may be estimated and may give some indication as to the severity and prognosis of acute and chronic parenchymatous liver disease. Total serum protein investigations give little practical information. Similar conclusions were reached by Higgins *et al.* (1944). A more satisfactory picture is given if these investigations are repeated at weekly intervals during the course of the illness. In the non-jaundiced subject the biochemical methods enumerated have proved of little value.

Summary

1. Sections of liver obtained by aspiration biopsy were studied in 187 patients with liver disease. Four groups of cases were investigated :—acute hepatitis, cirrhosis, obstructive jaundice and miscellaneous. Acute hepatitis was subdivided into four grades representing progressive decrease in the number of surviving hepatic cells. The cirrhoses were divided into active and inactive cases.

2. The histological appearances were correlated with the results of tests for serum bilirubin, alkaline phosphatase, total cholesterol and total and differential proteins, and with the intravenous hippuric acid and intravenous galactose tests.

3. In acute hepatitis the extent of the hepatic damage was reflected in the serum bilirubin and serum albumin levels. Galactose tolerance was impaired only in the more severe grades of damage. Impairment

of hippuric acid synthesis was inconstant and did not agree with the extent of the liver lesion.

4. The rapid recovery of the liver after hepatitis was reflected in a rapid fall of serum bilirubin and galactose time. Total serum protein and serum albumin showed a slight rise, serum globulin a slight fall. Recovery of normal hippuric acid synthesis often followed weeks after complete recovery of liver structure. Serum phosphatase and cholesterol fluctuated during the recovery period.

5. In histologically inactive cirrhosis no constant biochemical abnormalities were noted.

6. In active cirrhosis abnormalities were demonstrated by all the biochemical methods used. In ... ... ing of galactose time could ... ... hepatic cirrhosis; the findings by other methods bore little relation to histological changes.

7. In obstructive jaundice, galactose tolerance and hippuric acid synthesis were impaired and serum proteins decreased, thus reducing the differential diagnostic value of these tests. Serum phosphatase estimations proved of practical value in the diagnosis of primarily intrahepatic jaundice from that occurring secondarily to extrahepatic biliary obstruction. Only the intravenous galactose tolerance test reflected the extent of the hepatic damage found in association with obstruction to the common bile duct.

8. The miscellaneous group without jaundice showed no biochemical abnormality apart from an occasional rise in phosphatase or a low excretion of hippuric acid.

9. For the routine study of jaundiced patients the most valuable and practical tests are those for serum bilirubin and phosphatase and differential protein estimations repeated at weekly intervals.

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A CASE OF A HITHERTO UNDESCRIBED LIPOIDOSIS SIMULATING RHEUMATOID ARTHRITIS

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(PLATES LXXXIX-XCIV)

THE presence of nodules in association with rheumatoid arthritis has been described by a number of observers (*lit.*, Collins, 1937 ; Bennett *et al.*, 1940 ; Parkes Weber, 1943 ; 1944 *a* and *b*). Clinically the outstanding features of the present case were : (1) a polyarthritis which was initially of rheumatoid type but later developed atypical features ; (2) multiple cutaneous, subcutaneous and intramuscular nodules of xanthomatous type, in one of which a sarcoma ultimately developed. In addition to the gross lesions which were manifest clinically or radiologically during life, a remarkable microscopical lipoid infiltration of voluntary muscle fibres was observed after death.

Although there are features of this case which appear to be unique we feel that there is some justification for regarding the disease as one of the lipoidoses. Tendon, bone and joint lesions are met with in several diseases in this group. Thannhauser and Magendantz have usefully subdivided " primary essential xanthomatosis " (cholesterosis) into hypercholesterolaemic and normocholesterolaemic types (Thannhauser, 1940). A persistently high serum cholesterol is found in xanthoma planum et tuberosum of the skin, tendon xanthoma, xanthomatous biliary cirrhosis and xanthomata of blood vessels and endocardium. These lesions are commonly found together, but each may occur as a distinct entity. On the other hand, the serum cholesterol shows a normal or at most a high normal figure in xanthoma disseminatum, osseous xanthoma and the Schüller-Christian syndrome, and in xanthomatous involvement of the lungs, spleen and lymph nodes. In spite of the very close similarity of the histological picture in the two categories, the clinical distinction between them is clear cut and intermediate varieties appear to be rare indeed.

Tendon xanthemata generally show a familial incidence. They are found particularly in sites subjected to pressure and over bony prominences, for example the tendo Achilles, knuckles and knees. The xanthomatous nodules, inseparable from the tendons in which

they arise, are often whitish in colour, but are generally found in association with yellow skin lesions—xanthelasma of the eyelids and xanthoma planum et tuberosum of the skin. A high blood cholesterol is regularly present. Associated bone and joint changes have not been described, though a tendon xanthoma may cause disturbance of function of a joint by virtue of its situation.

Two cases of polyarthritis associated with xanthomatosis and hyper-cholesterolæmia have been recorded. The first was demonstrated by Parkes Weber and Freudenthal (1936-37) at the Royal Society of Medicine as a case of " nodular non-diabetic cutaneous xanthomatosis with hypercholesterolæmia " and a full account has since been published (Parkes Weber, 1944a). The patient, a man of 35, first complained of pain and stiffness in a number of joints, soon followed by the appearance of multiple red cutaneous nodules, situated chiefly in the region of joints and over bony prominences. There were no X-ray changes in bones or joints. Biopsy of the nodules showed them to be composed of aggregations of large, multi-nucleate " pre-xanthoma cells ", so-called because they contained only traces of demonstrable lipoid in their copious clear cytoplasm. There was a somewhat raised serum cholesterol (highest figure 350 mg. per 100 c.c.), but little else of note. On a fat-poor diet the serum cholesterol fell, the patient's condition improved and the nodules progressively diminished in size. When seen in 1943—seven years after the onset—he had almost recovered functionally, though arthritic deformities persisted.

The second case was described by Layani (1939 ; Layani *et al.*, 1939) under the title of " xanthomatous chronic deforming rheumatism ". The patient was a woman of 46 when first seen in 1936, but her illness began some fifteen years earlier. The whole duration was seventeen years, during which there were three phases in the joint condition : (1) a short acute phase lasting six months, when the arthritis was monarticular and resembled gout, followed by a short remission, (2) a prolonged phase lasting about fourteen years, characterised by a subacute polyarthritis with pain, stiffness, progressive loss of function and finally ankylosis of joints, (3) a terminal phase lasting two or three years in which there was progressive destruction of long bones, chiefly in the juxta-articular regions, by xanthomatous tissue. This bone destruction led to remarkable deformities, with abnormal mobility of joints and multiple spon-taneous dislocations. In the later stages recognisable manifestations of primary essential xanthomatosis of hypercholesterolæmic type appeared—xanthoma planum et tuberosum, cardiac angina of effort progressing to angina at rest, and persistent painless jaundice which was diagnosed as xanthomatous biliary cirrhosis. The serum total cholesterol reached the high figure of 1344 mg. per 100 c.c. a few months before her death early in 1938. There was no post-mortem examination.

A third case of rheumatoid arthritis with xanthomatous nodules, mentioned by Parkes Weber (1944a), has now been published in detail by Fletcher (1946). This patient had a normal blood cholesterol and from a study of the photo-micrographs it appears to us that the histological picture of the nodules does not differ fundamentally from that of the classical rheumatoid arthritis nodule (Collins ; Bennett *et al.*). Clinically the case differed from straightforward rheumatoid arthritis only in the abundance of the cutaneous nodules.

We have been unable to trace in the literature reports of any other cases in which polyarthritis was associated with xanthomatous nodules and it would appear that the syndrome is exceedingly rare.

The case here presented differs in several fundamental respects from those of Parkes Weber and Layani. Our case, like Layani's,

showed extensive juxta-articular destruction of bone by xanthomatous tissue, resulting in deformities of joints, but in Layani's case cutaneous and subcutaneous nodules were absent and in the terminal stages other manifestations of hypercholesterolæmic xanthomatosis made their appearance. The serum cholesterol was normal in our patient. Parkes Weber's case presented cutaneous xanthomatous nodules, but no bone changes were detected radiologically and the course of the disease was essentially benign, apparently responding to treatment by low fat diet. In our case the course was unaffected by any form of treatment. In both Parkes Weber's case and our own a remarkable increase of the skin nodules occurred on exposure to heat.

In the normocholesterolæmic type of primary essential xanthomatosis osseous xanthomata are well known, but these patients usually develop diabetes insipidus and exophthalmos due to lesions of the dura and base of the brain (Schüller-Christian syndrome). A feature of these bone deposits is that they always involve the membrane bones of the skull in addition to the long bones, pelvis, etc.; furthermore there is no predilection for the juxta-articular regions of the long bones. Our case showed no macroscopic deposits in the skull.

As regards the bone and joint changes which may be met with in the other lipoidoses, mention should be made of the extensive marrow involvement in Gaucher's disease. Subacute arthritic attacks have been described, which apparently arise from bone destruction in the neighbourhood of joints, and spontaneous fractures have also been recorded (Thannhauser). It should be noted that the joints are affected secondarily to the bone marrow. In our case there was no systematised involvement of the reticulo-endothelial system such as is encountered in Gaucher's disease. Niemann-Pick's disease has only been met with in early infancy and has little in common with the present case.

The changes in voluntary muscle noted in our case appear to be unique and are of fundamental importance if the case is to be regarded as an example of a primary lipoidosis—that is, of a disturbance of the intracellular lipoid metabolism which is not secondary to hyperlipæmia. In the hitherto described primary lipoidoses, the lipoid droplets always appear initially in the cytoplasm of histiocytes or reticulum cells, though in the later stages these cells often disintegrate, leaving deposits of the free lipoid in the tissue spaces. According to Thannhauser's conception, diseases of this class may be regarded as "metaplastic histiocytic and reticular lipidoses", that is, the underlying metabolic disturbance is inherent in the cells of the reticulo-endothelial system and in no other cells. The changes present in voluntary muscle in our case suggest a primary involvement of muscle fibres, as well as, perhaps, the histiocytes of the connective tissues.

In a recent paper, Steiner *et al.* (1946) described focal inflammatory and degenerative lesions of skeletal muscle occurring in cases of

rheumatoid arthritis. Lesions of this type were not met with in our case.

It is impossible on the evidence of a single case to assess the significance of the sarcomatous growth which developed in our patient. We can only state that the tumour appeared to originate in one of the symmetrical subcutaneous nodules.

Case report

Clinical history

The patient, H. I. aged 20, a barman, served with the Army in France in 1939. He was an only child of non-Jewish parents and there was no family history of any similar disease.

The first symptoms were slight stiffness and pain in the knees and ankles in December 1939, while living under damp conditions. He did not notice any swelling of the joints or fever, and denied the presence of any urethral discharge. At the end of December he noticed a small lump on his scalp while combing his hair. On 20th January 1940 he was admitted to the 3rd General Hospital, B.E.F., with a diagnosis of rheumatic fever and a doubtful lesion of his mitral valve. He was transferred to Friern Hospital in February 1940. On examination he was well developed and did not seem ill. He complained of pain and stiffness of the fingers and wrist; the pain and stiffness of the knees and ankles had almost ceased. There were a few small nodules on the scalp. By March 1940, symmetrical peri-articular swellings of the wrists and fingers, like those of rheumatoid arthritis, had developed but the movements of the joints were little affected. Some nodules were now present in the skin of the forehead and a few were noticed on the fingers. About this time he complained of stiffness of the shoulders and elbows, but swellings of these joints were not discovered. In May 1940 tonsillectomy was performed but no improvement in the joint condition followed. The state of the teeth and gums was satisfactory. The stiffness of the hands steadily increased, despite a full course of Myocrisin, and by July 1940 there was no movement at the phalangeal joints. The hands could not be brought up to the mouth and the forehead could only be touched when it was bent forward. The ankles and knees were unaffected. Small nodules were now very numerous all over the scalp, with two big ones on the back of the head. These, like all the other big nodules which subsequently appeared, were symmetrically placed. There were many superficial nodules on the face and side of the nose (fig. 1). Very large nodules, some 2 inches in diameter, were present over the site of the olecranon bursa, similar to those which appear in advanced gout. There were also subcutaneous nodules round the wrists and over the flexor tendons of the fingers (fig. 2).

In June 1940 a second course of Myocrisin was started and 0·9 g. given in all. Artificial fever was induced by means of short waves produced by an Inductotherm apparatus (Brodribb, 1937) and eight such treatments were given in the next 2½ months, with a temperature of 105° to 106° F. for eight hours; they caused no undue distress. The sedimentation rate, which had previously varied between 7 and 9 per cent., now rose to 16 per cent. and it remained about this level in spite of the pyrogenic treatment. A striking improvement took place in the joints of the hands and elbows, which enabled him to bring his hands to his mouth. The movements of the scapulae were much limited and did not allow any rotation. For the rest of his life, despite all treatment, the joints hardly varied in their range of movement. During the period of fever treatment a diminution of the nodules on the scalp was observed, but elsewhere the nodules increased greatly in size and number. It was thought at the time that the fever, which had benefited the

joints so much, was responsible for the increase of the nodules. A large, mobile nodule was present deep in the subcutaneous tissue on each side of the neck, opposite the middle of the sterno-mastoid. These on the elbows and wrists and along the tendons of the fingers were much larger, and some were present on the terminal phalanges. The nodules on the upper part of the back were confluent, forming two wide ridges over the scapulæ (fig. 3). There were large nodules over the posterior superior iliac spines and over each ischial tuberosity, which made the sitting posture uncomfortable. On the front of each thigh, about 10 cm. above the knee, there was a large nodule, about 6 × 4 cm., attached to the quadriceps muscle. During the course of the next few months many of the nodules diminished in size again.

Histology. In March 1941 one of the quadriceps nodules was removed by Mr J. B. Hume, who reported on the way in which it burrowed down in the muscle, its attachment to all the fascial planes at first suggesting a malignant tumour. Professor Hadfield reported as follows :—Two ovoid nodules were received for examination. One measured 1·3 × 0·7 × 0·4 cm., the other 1·2 × 1·75 × 0·5 cm. Both were soft, greyish white and homogeneous. The smaller was divided into two rather indistinct lobules, the larger into three. In paraffin sections the tumours are found to consist of large closely packed cells of one type held together by a minimum of connective tissue (fig. 8). The cells are ovoid to spherical but, being closely packed, many have become polyhedral from mutual pressure. The cells vary from 10 to 36 μ in diameter, average about 25 μ, larger cells predominating. The outer limiting membranes are precisely stained and each cell is well defined. The majority of the cells contain a cyto-reticulum giving the appearance of a finely dispersed cytoplasmic emulsion. This is incomplete in a fair number of cells ; in a few it is absent. The nuclei appear small in comparison with the cell volume, their diameter being rather less than a quarter of the cell diameter. They are centrally or eccentrically placed and very few are marginal. The majority have a clearly defined nucleolus, an ill-defined granular chromatin network and a delicate but heavily stained nuclear membrane. There are no multinucleate cells. The connective tissue of the nodules is scanty and consists of fine fibroglia in association with large fibroblastic cells. There is no fully formed collagen. The blood vessels are of capillary dimensions and well formed. The edges of the nodules merge into the surrounding subcutaneous connective tissue without any suggestion either of capsule formation or of infiltration. Frozen sections stained by fat stains give negative results. Unstained frozen sections show no double refraction in polarised light. The cells of the nodules contain no mucin as judged by mucicarmine staining, and no glycogen. The appearances in paraffin sections are identical with those in the xanthomata. As most of the cells had " foamy " cytoplasm it was expected that they would give a micro-chemical reaction for fat or lipoid. The micro-chemical tests so far carried out throw no light on the nature of the substance which occupies the meshes of the cyto-reticulum.

The cholesterol content of another nodule was determined. When cleaned the tumour weighed 1·88 g. and it contained 3·3 mg. (or 0·18 per cent.) of cholesterol, which is an average concentration for normal tissues. The figures for the serum cholesterol at this time were :—total cholesterol 170 mg., free cholesterol 54 mg., cholesterol ester 116 mg. per 100 c.c. The blood urea was 30 mg. and the blood uric acid 4 mg. per 100 c.c.

In March 1941 pain and swelling of the jaw was noticed, and Mr J. D. Cambrook made the following note : " The change in the condition of the patient's teeth during the last ten months is truly remarkable. His teeth showed no infection then, but now there is evidence of ascending infection around each tooth, which in some cases has reached the apex and produced quite large areas of rarefaction. All teeth are loose and mastication must be quite difficult ". Sepsis followed extraction of all the teeth and Mr Cambrook then reported : " I have never before seen anything quite like this, because the blood clot does not break down and disintegrate as normally happens in a septic socket, but remains as a grey mass of decolourised blood clot ". The sockets eventually healed, and the gums became firm and gave no further trouble. At this time the condition of the joints had improved sufficiently to allow the patient to get up, and he was able to walk with the aid of two sticks. The knees could not be completely straightened and the back showed a definite kyphosis. In June and July 1941, after sitting in the hot sunshine, many more nodules appeared on the scalp and on the part of the chest exposed through an open shirt. It was thought that this was due to the effect of the heat.

In August 1941 he was transferred to St Bartholomew's Hospital for deep X-ray therapy to the left side of the forehead and left hand. Six treatments of 100-400 skin doses were given to the left arm and the same number to the forehead without causing any alteration in the nodules or joints. At this time the fingers became hyper-extended and splints were applied to maintain flexion. In September 1941 a change in the sternum was noticed ; it gradually became depressed and, in time, the front of the chest seemed to fall in. He complained of tenderness over the lower ribs and along the spine. The X-ray pictures of the hands and wrists at this time showed peculiarly roughened bony outlines and there were large erosions on the heads of the metacarpals and most of the phalanges. In December 1941 the rounded areas of destruction were more numerous and more obvious. In May 1942 new nodules appeared on both sides of the neck under the sterno-mastoid and the sternum was still more depressed. Hyperkeratosis of the back of the thighs, similar to that which occurs in scurvy, was noticed. This was the more remarkable as 100 mg. of ascorbic acid had been given daily for seven weeks (4900 mg.), but ascorbic acid was not being excreted in the urine in spite of this large dose. In September 1942 X-rays of the skeleton showed patchy destruction of bone in several vertebral transverse processes and in the heads of the ribs. The surface outlines of both tibiæ and of the metatarsals and proximal phalanges of the toes were indistinct and " roughened ".

At the end of October 1942 a nodule had appeared in the right axilla, which grew rapidly in size. This was the first large nodule which was not symmetrical in that it grew rapidly to a very large size until it bulged from the axilla. It was tender to touch and the colour of the overlying skin changed from red to a dusky purple. A brawny area developed in the outer part of the pectoralis major, without any pitting, while pitting œdema developed in the upper arm, extending down to the elbow. There was no anæmia and a blood count on the 24th November showed red blood cells, 5,500,000 per c.mm. and hæmoglobin

LIPOIDOSIS SIMULATING RHEUMATOID ARTHRITIS

FIG 1 —Photograph showing cutaneous
nodules on face

FIG 3 —Photograph showing sub-
cutaneous nodules on back

FIG 2 —Photograph showing nodules on fingers   The fingers are held
apart by pieces of cork

120 per cent. A leucocytosis of 17,500 per c.mm. was present. From early in December 1942 he was confined to bed owing to muscular weakness. On 13th December the surface of the tumour began to ulcerate at its most dependent part, and oozed a thick offensive fluid which yielded a mixed growth on culture. Radiograms taken on 29th December showed that the lungs were healthy, but there was bone destruction in the upper margin of the left scapula and periostitis of the shaft of the humerus and ulna on both sides. There was also some bone destruction in the lower end of the humerus and in the olecranon (figs. 4 and 5). By the end of January 1943 the axillary tumour was completely necrosed and liquefying. On 6th February 1943 the œdema had spread down the arm to the hand. On 8th February some diarrhœa occurred and he died next day.

Autopsy findings

External appearances. The body showed considerable emaciation. There was slight symmetrical pitting œdema of the feet. The knees, ankles, shoulders, elbows and wrist joints all showed marked symmetrical enlargement due to diffuse peri-articular swelling. There were fusiform peri-articular swellings of the small joints of the hands ; the interosseous muscles were wasted and the hands showed advanced ulnar deviation deformity. The front of the thorax was flattened and the centre of the sternum depressed owing to posterior dislocation of the body of the sternum at the manubrio-sternal junction. This joint also showed great thickening of the peri-articular tissues.

A large number of more or less discrete, rounded, nodular swellings were present in the skin and subcutaneous tissues of the scalp, face, trunk and limbs. The hairy part of the scalp was covered by innumerable small nodules. Many of the larger nodules were arranged in roughly symmetrical fashion and on the limbs they tended to be most numerous in the neighbourhood of joints and over bony prominences. On the trunk they were mainly concentrated on the back, where there was a diffuse, soft, subcutaneous " cushion " over the scapulæ. The nodules varied in size from a few mm. to 7 or 8 cm. in diameter (olecranon), the largest discrete nodules being situated on the upper limbs. In consistency they were very variable ; the smaller (for example those in the scalp, where they appeared to be in the cutis itself) were firm, whereas many of the larger ones (for example those on the upper arms) were subcutaneous and tended to be soft. The skin overlying some of the softer nodules was loose and redundant ; it showed no discolouration or evidence of nutritional disturbance. Some nodules were fixed to underlying fascia and muscle, but the majority were freely mobile. On section the smaller nodules were moderately well defined, somewhat opaque and whitish ; those of larger size were poorly defined and consisted of glistening, semi-translucent, gelatinous tissue.

Projecting from the right axilla there was a large, partly necrotic tumour over which the skin was discoloured and centrally ulcerated. This tumour was soft, opaque and hæmorrhagic, having the appearance

of a rapidly growing sarcoma. It measured approximately 12 cm. in diameter and extended up to the apex of the axilla, but, although not encapsulated, it was not fixed, either to the chest wall or to the humerus. A typical subcutaneous nodule was present on the thoracic wall in the left axilla.

Internal appearances. The subcutaneous tissues and muscles throughout were pale and œdematous. The subcutaneous fat was scanty and there was marked muscular wasting.

The tongue was normal; the teeth and tonsils were absent. In the pharynx a well defined nodule 1·5 cm. in diameter, similar in appearance to those on the exterior of the body, was situated beneath the mucous membrane on the posterior aspect of the cricoid cartilage. A discrete nodule was discovered in the substance of the infrahyoid muscles. Both of these nodules were opaque and pale pink on section. The larynx and vocal cords were normal. The cervical lymph nodes were somewhat enlarged and fleshy.

The trachea and bronchi were hyperæmic. The œsophagus was normal. On the right side there were dense pleural adhesions anteriorly. Posteriorly there was a loculated empyema containing thin, bloodstained pus. The parietal pleura was thickened and grossly œdematous. On the left side there were a few fibrous adhesions over the posterior and inferior aspects of the lung. In the right lung the lower lobe was partially collapsed and almost airless. The upper lobe was œdematous but not consolidated. The left lung was œdematous and congested but not consolidated. Macroscopic metastases from the axillary tumour were not found in the lungs or elsewhere in the body.

The pericardium contained about an ounce of clear, straw-coloured fluid. There were no adhesions between the layers, but the serous surfaces of both showed numerous irregular patches of hyaline pericarditis. The heart showed no enlargement. The myocardium was pale. There was a series of small bead-like swellings along the free edge of the mitral valve, which, however, did not show generalised thickening or scarring suggestive of rheumatic infection. The other valves were normal. The coronary vessels were normal. The abdominal aorta showed very early atheroma.

The peritoneum contained a few ounces of clear fluid. The stomach and intestines were normal. The mesenteric lymph nodes were all somewhat enlarged and fleshy. The liver was somewhat enlarged (1900 g.). Its consistency was firm and the cut surface showed exaggeration of the normal lobular pattern. Enlarged fleshy lymph nodes were present in the portal fissure. The gall bladder and bile ducts were normal. The spleen was slightly enlarged (400 g.). Some delicate flakes of fibrin were adhering to the outer surface of the organ. Two small accessory spleens were present. The cut surface showed prominent Malpighian bodies. The pulp was soft and deep red in colour.

PLATE XC

LIPOIDOSIS SIMULATING RHEUMATOID ARTHRITIS
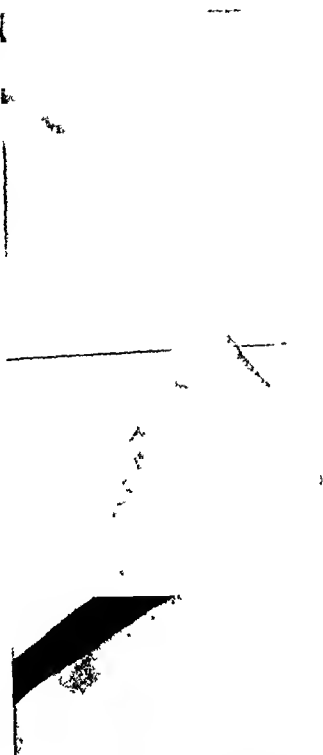
Fig. 4—Radiograph of left elbow joint (1942) The dense shadow is due to a large nodule

Fig 5—Radiograph of right elbow and humerus (1942), showing nodule on elbow and large swelling in axilla The opaque spots are due to the iodoform which had been powdered on the tumour to mask the unpleasant smell of the ulcerating neoplasm

The kidneys were congested but showed no other macroscopic changes : bladder and prostate were normal.

The calvarium was heavy but otherwise normal. The pituitary fossa was normal in size. There was slight thickening of the lepto-meninges. The brain appeared normal. The pituitary, thyroid, parathyroids, thymus and suprarenals showed no macroscopic abnormality.

There was a rounded kypho-scoliosis in the lower thoracic region with a compensatory lumbar lordosis. Most of the bones appeared normal save in the neighbourhood of joints ; no fractures were present. The bone marrow of the femoral shaft showed the appearances of gelatinous degeneration, mottled by small patches of red marrow.

All the joints examined showed peri-articular thickening, with much œdema of the capsule. In several joints (notably the manubrio-sternal junction) there was considerable laxity of the peri-articular ligaments, allowing an abnormal range of movement. The large soft right olecranon nodule showed on section a small cavity in its deeper part, filled with thin purulent fluid. The appearances suggested an inflamed and thick-walled bursa. The joint cavity contained a small effusion of clear fluid. The synovium was thickened and grossly hyperæmic. The cartilages of the olecranon and humeral condyles were extensively and irregularly eroded, exposing the underlying bone, which was very hyperæmic and much rarefied.

Histological findings

Sections were taken from several of the cutaneous and subcutaneous nodules, the intramuscular nodules in the neck, the right axillary tumour, the right olecranon bursa, portions of voluntary muscle selected at random, portions of bone, including joint surfaces, the femoral bone marrow, portions of the right lung and pleura, the heart wall, liver, spleen, pancreas, an enlarged lymph node from the portal fissure, and the endocrine glands. Paraffin sections were stained routinely with hæmatoxylin and eosin ; selected blocks were also stained by Mallory's aniline blue method, Gram's method, Congo red, and Best's carmine stain. Frozen sections of the nodules and voluntary muscles were stained by Scharlach R. In nearly all these tissues the microscopical appearances are modified by post-mortem autolysis and in some there is evidence of a terminal septicæmia.

The sections show extremely widespread infiltration of certain mesodermal tissues by large free cells, the majority of which have " foamy " cytoplasm. For the sake of convenience these cells will be termed xanthoma cells, although the chemical nature of the cytoplasmic droplets is unknown. A detailed description of these cells has already been given in the biopsy report. It may be added that the xanthoma cells in many different areas all show the same essential features ; only minor variations are found. The great majority are mononuclear, though multinucleate cells of identical appearance but containing six or more nuclei are found in the skeletal muscles. Cells in mitosis are not found. The size of the xanthoma

cells is variable, and in some sites small cells with a relatively opaque, granular cytoplasm predominate. However, when such cells are examined under the oil immersion objective, the cytoplasm is seen to be distinctly foamy. This appearance naturally suggested the presence of some lipoid substance, but Scharlach R staining of frozen sections gave negative results in all tissues except voluntary muscle. Even here anisotropism was only demonstrable in some of the cells. Stains for glycogen and amyloid gave completely negative results. The widespread distribution of these cells is remarkable, for they are interspersed throughout the dermis and subcutaneous fat, the voluntary muscles, fascial planes, periosteum and synovia. The superficial and deep nodules are composed simply of dense aggregations of xanthoma cells, sometimes arranged in strata or laminæ (figs. 6 and 7). There is no clearly defined edge to most of the nodules, as the xanthoma cell infiltration tails off towards the periphery. In some of the scalp nodules many of the xanthoma cells have disintegrated, leaving deposits of free lipoid in the tissue spaces. In most areas the xanthomatous infiltration is associated with remarkably little inflammatory reaction, but a minor degree of capillary and fibroblastic proliferation is observed in the subcutaneous deposits and the reaction in the synovia and bones is more vigorous. There is very little fully formed collagen in the nodules and necrosis is not observed. Their structure thus differs fundamentally from that of the classical necrobiotic nodule of rheumatoid arthritis. Proliferation of fibrous tissue in the intima of small arteries is observed in some areas.

Skin and subcutaneous tissues. In relation to the small nodules of the scalp, the xanthoma cells are grouped together in the sub-papillary layer of the dermis. The overlying epidermis is attenuated and small groups of xanthoma cells have insinuated themselves into the epidermis itself. In relation to the larger nodules (hand and scapular region) they are located in the subcutaneous tissues, and in heavily infiltrated areas the subcutaneous fat appears to have been absorbed.

Voluntary muscles. Sections were taken from the pectoralis major, brachialis and infrahyoid muscles, post-cricoid muscles and diaphragm. Similar changes are seen in all sections, but the severity of the muscle degeneration varies in different regions. The most advanced changes are found in the diaphragm, where no normal fibres remain. The essential lesion consists in a peculiar lipoid infiltration of individual muscle fibres, which is associated with the presence of "xanthoma cells" (fig. 10). The muscle fibre degeneration is apparently haphazard in its distribution, picking out small groups of fibres, individual fibres and even part of a single fibre here and there throughout the muscle; the intervening fibres show little alteration. The sequence of changes in a degenerating fibre seems to be as follows:

(i) Minute clear droplets appear in the sarcoplasm between the myofibrils (fig. 11). A certain proportion of these droplets stain red with Scharlach R.

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Fig. ... ... all subcutaneous nodule from dorsum of hand,
all infiltration tailing off gradually at the periphery.
small arteries   ×12

Fig 7.—Photomicrograph of post cricoid intramuscular nodule, showing its
general topography.   ×5 5.

LIPOIDOSIS SIMULATING RHEUMATOID ARTHRITIS
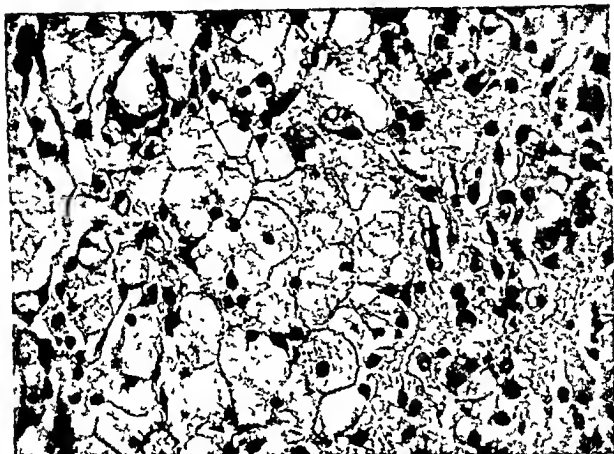
FIG 8 —Photomicrograph of biopsy nodule from quadriceps stained with hæma toxylin and eosin, showing the characters of the xanthoma cells ×320
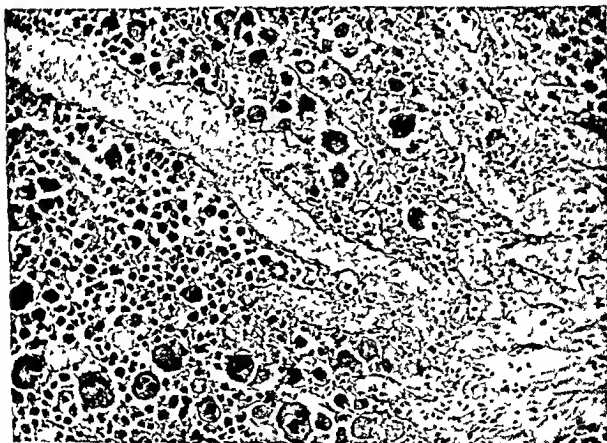
FIG 9 —Photomicrograph of tumour from right axilla showing the polymorphic character of the tumour cells the appearance of the vessels and an area of necrosis (top right) ×130

(ii) With progressive accumulation of lipoid, the droplets coalesce and the myofibrils break up and disappear. The sarcolemma remains intact as a sheath enclosing rows of large clear globules or a " foam " of minute droplets (figs. 12 and 13). The nuclei of the muscle fibre persist until a late stage but often show pyknosis.

(iii) Ultimately the distended sarcolemma disintegrates, releasing the lipoid globules. Frozen sections of muscle show variable amounts of Scharlach-positive material in muscle fibres, some of it free in the tissue spaces or contained within histiocytes. Thus the pectoral muscle, in which fibre degeneration is fairly advanced, contains considerable quantities of fat, some of it anisotropic. A proportion of the xanthoma cells in muscle, as elsewhere, contain cytoplasmic droplets which are Scharlach-negative and isotropic; the intramuscular post-cricoid nodule, for example, fails almost entirely to take up the fat stain, yet the cells of which it is composed are otherwise identical with the xanthoma cells in other muscles. The histological appearances suggest an intimate relationship between these cells and the degeneration of muscle fibres. Xanthoma cells are not infrequently seen lying inside the endomysial sheaths of individual fibres and a few are contained within an apparently intact sarcolemma.

Heart muscle and non-striated muscle. The changes observed in the skeletal muscles are absent in the myocardium and in smooth muscle. Xanthoma cells are not present in those situations.

Bones and joint surfaces. Sections were taken, after decalcification, from the lower end of the humerus (right arm), including the joint surface, the olecranon of the right ulna, the head of the right radius and a costo-chondral junction from one of the middle ribs. All sections show simple rarefaction of the cancellous bone lamellæ. In addition there is active destruction and absorption of bone by a highly vascular granulation tissue containing numerous xanthoma cells. This change is confined to the cortical bone actually in contact with synovial membrane or periosteum and much the most severe changes are met with in the articular regions. The thick layer of granulation tissue which covers the articular surfaces, eroding and undermining the articular cartilage, is richly infiltrated by xanthoma cells (fig. 14). Small numbers of leucocytes are also present and some of the small arteries enveloped by this tissue show eccentric obliterative endarteritis. The *bone matrix* in contact with this tissue shows irregular absorption in the early stages, apparently due to the activity of xanthoma cells, since typical osteoclasts are absent. As the granulation tissue encroaches on the underlying bone, the eroded lamellæ become isolated as microscopic sequestra surrounded by xanthoma cells and lying in a matrix of fibrous tissue (fig. 15). In the case of the costo-chondral junction, the rib is separated from its cartilage by a layer of granulation tissue of identical type continuous with the perichondrium and periosteum on the surface. Irregular absorption of the bony and cartilaginous matrix lying in contact with

this tissue is again observed. The abnormal laxity and mobility of synchondroses (notably the manubrio-sternal junction) may well have been due to the penetration of all the osteo-chondral junctions by vascular granulation tissue containing xanthoma cells. A similar mechanism probably caused the loosening of the teeth, predisposing to periodontal infection. The cortical bone at varying distances from the joints shows patchy erosion by xanthoma cells on the deep surface of the periosteum. New bone formation is not conspicuous in any of the sections. The medullary cavity of the bones is normal.

Olecranon bursa. Sections show acute suppurative bursitis; the purulent exudate in the sac contains numerous streptococci. The bursa is lined by œdematous granulation tissue sparsely infiltrated by xanthoma cells.

The right axillary tumour. Sections from three different regions of the tumour all show the structure of a polymorphic-celled sarcoma (fig. 9). The tumour is almost entirely necrotic and heavily infected, so that finer cytological details are obscured.

Lungs and pleura. Sections from the right lung base show a chronic streptococcal empyema with considerable œdema and vascularity of the pleural layers. The lung parenchyma shows acute perivascular and peribronchial lymphangitis. A few xanthoma cells lie free in the alveoli; there is no pulmonary fibrosis.

Femoral bone marrow. A relatively acellular marrow, showing gelatinous replacement of the fat. There is no great pathological alteration in the cytology, and a slight preponderance of the myeloid leucocyte series could be explained by the leucocytosis of infection. A very occasional xanthoma cell is seen.

Spleen. The splenic architecture is normal. The cells lining the engorged sinusoids appear normal, as do the vast majority of the histiocytes in the pulp, though polymorphonuclear leucocytes are present in excess. Occasional xanthoma cells are seen, in both sinusoids and pulp.

Lymph node. The enlarged lymph node from the portal fissure shows acute lymphadenitis. The pulp is œdematous and the dilated lymph sinuses contain large numbers of polymorphonuclear leucocytes. In addition, there are fair numbers of xanthoma cells in the sinuses and a few in the pulp.

Liver. The appearances are those of passive venous congestion, with fatty changes. There is no evidence of increased activity of the Kupffer cells, nor is there any significant increase of cells in the portal tracts.

Pancreas. Sections show nothing abnormal.

Kidneys. In a small proportion of the glomeruli, one or more of the capillary loops composing the tuft are swollen and pale-staining. Examination of these under the $\frac{1}{6}$th in. objective reveals a clump of xanthoma cells impacted in the capillary loop. The remaining glomeruli appear normal, but around some there are focal collections

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FIG. 10.

FIG. 11.

FIG. 12.

FIG. 13.

FIG. 10.—Photomicrograph of infrahyoid muscle, showing degeneration of muscle fibres and " xanthoma " cell infiltration.   × 45.

FIGS 11-13.—Photomicrographs of infrahyoid muscles stained by Mallory's technique, showing stages in the muscle fibre degeneration   × 260.

FIG. 11.—Small lipoid droplets are seen between the myofibrils of one fibre, while globules of larger size are present in an adjacent fibre. " Xanthoma " cells are seen in close relation to a fibre in the centre of the field.

FIG. 12.—Fibres in which the sarcoplasm is filled with a " foam " of lipoid droplets. Note " xanthoma " cells (top left).

FIG. 13.—Degenerate fibres showing distension of the sarcolemma by large globules. Traces of cross striation can still be seen.

PLATE XCIV

LIPOIDOSIS SIMULATING RHEUMATOID ARTHRITIS

FIG. 14.—Photomicrograph of head of radius, showing erosion of articular surface and cortical bone in neighbourhood of joint by a thick layer of xanthomatous granulation tissue. The marrow is not involved. The remains of the articular cartilage can be seen in the bottom left-hand corner of the field. × 6.

FIG. 15.—Photomicrograph of head of radius. The field is indicated in fig. 14. It shows microscopic sequestra enveloped by xanthomatous granulation tissue. × 90.

of lymphocytes and polymorphonuclear leucocytes. The epithelium lining the proximal convoluted tubules shows extensive hyaline droplet degeneration.

Endocrine glands. No significant alterations are found in the pituitary, thyroid, parathyroid or suprarenal glands, or in the islets of Langerhans.

SUMMARY

A case of a hitherto undescribed disease is reported. A well nourished young man, aged 20, with no significant antecedent history, developed a polyarthritis closely simulating rheumatoid arthritis in its symmetrical distribution and peri-articular swellings. The appearance of small cutaneous nodules coincided with the onset of the arthritis. As the disease progressed the nodules increased both in size and number, those of larger size being symmetrically disposed. Biopsy of a nodule showed the appearances of xanthoma but micro-chemical tests for fat, lipoid, glycogen and mucin were negative. The blood chemistry was within normal limits. Within six months there was severe disability owing to the arthritis. A year from the onset the teeth became loose and had to be extracted. The stiffness of joints and disability persisted until the end, but in the later stages the fingers became hyper-extended and abnormal laxity of the manubrio-sternal joint resulted in its dislocation. Radiograms of the skeleton revealed patchy bone destruction. Thirty-four months from the onset a nodule which had been noticed in the right axilla began to grow rapidly; fungation and ulceration followed. The terminal phase was marked by acceleration of the previously observed muscular wasting and general emaciation and he died three years and two months from the onset of the illness.

At autopsy, nodules were found in the skin, subcutaneous tissues and skeletal muscles; they were ill-defined, and either semi-translucent or whitish and opaque. The muscles were atrophic, pale and œdematous. The joints showed thickening and œdema of peri-articular tissues. The eroded articular surfaces were covered by thickened and hyperæmic synovium. The bone marrow showed "gelatinous degeneration". The spleen and lymph nodes showed slight enlargement. An empyema thoracis was present on the same side as the axillary tumour. No significant changes were found in other organs.

Histologically the essential lesion consisted in widespread infiltration of many mesodermal tissues (voluntary muscle, subcutaneous tissues, skin, synovial membranes and periosteum) by histiocytes with foamy cytoplasm. The chemical nature of the cytoplasmic droplets remained obscure, but there was evidence that a certain proportion of this material was either neutral fat or lipoid. In some situations these cells were so numerous and closely packed as to form nodules of macroscopic size. The presence of these cells in voluntary muscle was

related to a distinctive type of degeneration of the muscle fibres, characterised by the appearance of droplets in the sarcoplasm and ultimate disintegration of the fibres. Cardiac muscle and non-striated muscle were not affected. Infiltration of the synovial membrane and periosteum was accompanied by the formation of vascular granulation tissue and by destruction and absorption of bone in contact with this tissue. There was evidence that a few of these histiocytic cells had "overflowed" into the blood and lymph streams, but on the whole there was a striking absence of reaction on the part of the reticulo-endothelial system. The internal organs showed only minor changes and the endocrine glands were normal. The axillary tumour was an anaplastic, polymorphic-celled sarcoma.

It is suggested that this disease should properly be classed among the lipoidoses. A review is given of the muscle, bone and joint lesions occurring in known lipoidoses.

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SHORT ARTICLES

TURBIDIMETRIC ASSAY FOR PENICILLIN

C A GREEN

From the Royal Naval Medical School

A turbidimetric method for the assay of penicillin using *Staphylococcus aureus* as the test organism was described by Foster (1942) The possible usefulness of this test in plant control was claimed by Foster and Woodruff (1943), but the accuracy of the test was not entirely satisfactory. Foster and Wilker (1943) and Joslyn (1944) introduced further variations, including the use of culture tubes suitable also for direct turbidity measurements, but the two standard curves shown could not be exactly superimposed McMahan (1944) claimed that his modification of the turbidimetric test was both more rapid and more precise than the Oxford cup method (Heatley, 1944) A similar turbidimetric assay method has been used in this laboratory for two years for various purposes, including production plant control The test has proved exceedingly useful and reliable for this purpose

DETAILS OF TEST

Apparatus

(1) 6 × 1″ rimless test tubes, fitted with aluminium caps, chemically clean and sterilised in hot air oven at 160° C for 2 hours

(2) "Ayling" filler calibrated to deliver 20 ml

(3) 4 litre bottle with all metal two way head, autoclaved at 15 lb for 30 mins

(4) Graduated 1 ml and 10 ml pipettes sterilised in hot air oven at 160° C for 2 hours

(5) Volumetric pipettes 1, 2, 5, 10 ml etc

(6) Volumetric flasks 20, 50, 100 ml etc

(7) Water bath with circulator, thermostatically controlled at 37° C

(8) Photo electric absorptiometer

Reagents

(1) *Glucose broth medium* Evans peptone 50 g , marmite 15 g , Lab Lemco 15 g glucose (B D H , A R) 10 g, sodium chloride (B D H , A R) 35 g , dipotassium hydrogen phosphate (A R) 37 5 g , potassium dihydrogen phosphate (A R) 12 5 g , distilled water to 10 litres

Method of preparation Dissolve peptone, marmite, Lab Lemco, glucose and sodium chloride in approximately nine litres of distilled water Using 10 N sodium hydroxide, adjust to approximately pH 8 0 Steam at 100° C. for 30 mins Filter through Chardin papers Using concentrated hydrochloric acid, adjust to pH 7 0 Add buffer salts and make up the volume to 10 litres

Distribute in 1 litre and 100 ml. amounts. Autoclave at 15 lb. for 30 mins. The resulting broth should be crystal-clear at pH 7·0. Medium is stored in the dark at room temperature.

All the tests in any one series must be prepared from one batch of medium, which should be made in volumes sufficiently large to cover two or more weeks' work.

(2) *Chloroform phosphate buffer at* pH 6·2. 120 ml. $M/3$ disodium hydrogen phosphate (solution " A "); 480 ml. $M/3$ potassium dihydrogen phosphate (solution " B "); 3000 ml. saturated chloroform water.

(3) *Standard penicillin solutions.* A dried calcium salt of penicillin of known unitage is kept in a desiccator at room temperature. From this powder a solution of penicillin containing exactly ·1000 units per ml. is prepared by dissolving an accurately weighed amount in chloroform phosphate buffer. This is prepared once each week, serial assays showing that there is no deterioration over this period.

A " working standard " of 100 units per ml. in phosphate buffer is prepared from the above every 2nd or 3rd day, and from this a 10-unit standard in phosphate buffer is prepared daily. Immediately before use, this is diluted finally to 1 unit per ml. in glucose broth. Grade " A " N.P.L. glassware is used throughout.

(4) *Five per cent. formol-saline.*

Preparation of staphylococcal broth

The Oxford " H " strain of *Staphylococcus aureus* is maintained on agar slope subculture and agar plate subcultures are prepared daily. On the evening before the test a single colony is inoculated from the plate subculture into 100 ml. of glucose broth which is incubated overnight at 37° C. Next morning the purity of the glucose broth culture is checked· and the density determined by means of a Spekker absorptiometer, suitable calibration curves having been determined and allowance made for differences in unseeded batches of broth. The appropriate volume of glucose broth culture is added with sterile precautions to 4-litre volumes of glucose broth to give a final density of 5,000,000 organisms per ml.

Arrangement for determination of standard curve

The 1-unit·penicillin broth solution (prepared as in reagent 3 above) is distributed to 6″ tubes, fitted with aluminium caps to avoid gross contamination, as follows :—

Tube	1	2	3	4	5	6	7	8	9	10
1 unit penicillin broth standard .	Nil	ml. 0·1	ml. 0·2	ml. 0·3	ml. 0·4	ml. 0·5	ml. 0·6	ml. 0·7	ml. 0·8	ml. 0·9

By means of an Ayling filler, 20-ml. volumes of staphylococcal-seeded broth are added to each tube. Tubes 2-9 are put up in triplicate, *tubes 1 and 10* in quintuplicate.

Arrangement for determination of unknown solution

If the approximate strength of the solution under test is known, it is diluted to an approximate 10 units per ml. in phosphate buffer followed by a 1 : 10 dilution in broth. To each of 3 tubes is added 0·5 ml. of the 1 : 10 glucose broth dilution of the unknown solution, followed by 20 ml. of staphylococcal-seeded broth. If the strength of the solution to be assayed is unknown, it is necessary to make a series of falling decimal dilutions to cover the widest possible range.

The time required to complete the addition of staphylococcal broth to a full set of standard solutions and 60 unknown solutions is approximately 15 20 minutes This time should be kept as short as possible

Incubation

The tubes containing the standard and unknown solutions are spaced in every other compartment of wire racks arranged in a 37° C water bath at such a height that the tube contents are completely immersed

Under the conditions described, the standard tubes will show a suitable range of turbidity at approximately 4 hours Consequently the required end point is ascertained by determining the density in standard tubes 1 and 10 at intervals commencing at 3½ hours' incubation When the required density has been reached, the entire test is removed from the bath

Further growth is checked by the addition to each tube of 5 c c of 5 per cent formol saline No significant difference in results has been detected when tests are read immediately after the addition of formol saline or at intervals up to 3 hours later (longest period tested)

Reading of test Standard curve

The contents of the tubes are transferred serially to 25 ml capacity optical fused glass cells (4 cm in width) and the turbidity measured in a Spekker absorptiometer, using a Cambridge spot galvanometer and H503+H508 filters A Miller photo electric colorimeter (Morris, 1944) fitted with a constant 6 voltage transformer has proved as reliable and is speedier in operation

The means of the absorptiometer readings of the triplicate tubes containing the same amount of standard penicillin are plotted against the volume of penicillin contained in the tube

Determination of unknown

The mean of the three tubes containing the unknown solution is taken and the corresponding strength of penicillin is determined from the standard curve An example is shown in table I

TABLE I

Spekker and equivalent graph readings on sample of crude brew

Tube	1 50 dilution of unknown	Spekker reading	Graph reading (units/ml)
1	0 5 ml	0 26 ⎫	
2	0 5 ,,	0 26 ⎬ 0 26	0 600
3	0 5 ,,	0 26 ⎭	

A concentration of 0 5 ml of a 1 50 dilution of the unknown in a total culture volume of 20 5 ml yielded the same final opacity as a concentration of 0 6 ml of 1 unit penicillin solution in a total culture volume of 20 6 ml Therefore the neat unknown solution contained $\frac{0\ 6}{20\ 6} \times \frac{20\ 5}{0\ 5} \times 50$ units per ml $= 59\ 7$ units per ml

Shape of curve The shape of the standard curve tends to be sigmoid In preliminary trials it was the practice to test unknown solutions over the full range used for the standard curve, using duplicate or triplicate tubes at each

level. It was found that the shape of the curve was identical for approximately 10-unit dilutions of crude brew, acetone eluates, weak and concentrated sodium or calcium salts and solutions of dried preparation. For this reason it was decided that in a triplicate tube test it was preferable to use the same volume, namely 0·5 ml., of diluted unknown in each tube, rather than a range such as 0·4 ml., 0·5 ml. and 0·6 ml., thereby reducing the risk of pipetting errors.

Accuracy of test

(1) *Replication*. In a series of unknowns the usual triplicate arrangement was extended to a larger number of tubes. Examples of the range of assay results based on a single tube and on the usual triplicate tube reading were as in table II.

TABLE II

Replicate results by single and triplicate tube tests on two sample fluids

Tube	Result (units/ml.)		Tube	Result (units/ml.)	
	Single	Triplicate		Single	Triplicate
1	61·2 ⎫		1	1532 ⎫	
2	65·4 ⎬	63·6	2	1512 ⎬	1512
3	64·2 ⎭		3	1492 ⎭	
4	64·2 ⎫		4	1492 ⎫	
5	64·2 ⎬	62·8	5	1512 ⎬	1505
6	60·1 ⎭		6	1512 ⎭	
7	64·2 ⎫		7	1512 ⎫	
8	64·2 ⎬	63·2	8	1512 ⎬	1512
9	61·2 ⎭		9	1512 ⎭	
10	61·2 ⎫		10	1520 ⎫	
11	61·2 ⎬	61·6	11	1492 ⎬	1501
12	62·4 ⎭		12	1492 ⎭	
Highest . .	65·4	63·6	Highest .	1532	1512
Lowest . .	60·1	61·6	Lowest .	1492	1501
Mean . . 62·8			Mean . . 1507		

(2) *Reproducibility*. Repeat assays on successive days on a random group of routine samples (held at approximately 10 units per c.c. in phosphate buffer at 4° C.) resulted as in table III.

TABLE III

Repeat assays on random routine samples

		Oxford units per ml.	
Crude brew 546 . .	Diluted 1 : 50	57·4	55·7
,, ,, 548 . .	,, 1 : 60	65·8	63·0
Corn steep assay . .	,, 1 : 40	17·6	19·8
Acetone eluate . .	,, 1 : 70	63·0	65·1
Calcium salt C1 . .	,, 1 : 2000	2650	2500
,, ,, 534 . .	,, 1 : 2000	2820	2780
,, ,, 537 . .	,, 1 : 2000	2160	2120
,, ,, 546 . .	,, 1 : 4000	3040	2760
Sodium salt 544 . .	,, 1 : 2000	1460	1560
,, ,, 547 . .	,, 1 : 2000	1980	1888
Concentrated seitzed 528 .	,, 1 : 50,000	44,500	42,000
Dried salt C1 . .	,, 1 : 100,000	87,600	84,000

Assays repeated on successive days on a dried calcium salt for strict determination of potency for use as a laboratory substandard resulted as follows —

376	368	356	352	356
368	388	356	352	320
368	384	356	348	328
370	376	368	348	368
376	368	368	344	308
376	368	356	344	336
336	368	384	348	328
352	368	376	344	360
336	376	364	348	376
372	376	348	356	360
372	364	344	360	368

Using the mean of the above results the potency of the substandard salt was fixed at 360 units per mg

Estimation in different dilutions

Examples of assays at different dilutions on the same samples were as in table IV

TABLE IV

Results of assay at various dilution levels

Sample	Final dilution	Result (units/ml.)	Sample	Final dilution	Result (units/ml.)
Crude brew	1 30	57 0	Crude brew	1 30	62 5
	1 50	55 5		1 50	60 0
	1 70	54 0		1 70	61 0
Calcium salt	1 6000	6720	Seitz concentrate	1 50 000	61,000
	1 8000	7120		1 70 000	63,700
	1 10 000	7500		1 100,000	60,500

COMMENT

The addition of 0 1 per cent glucose to the culture broth has not detracted from the reliability of the test Different batches of broth tended to reach the desired maximum turbidity in slightly different periods and to yield standard curves with variations in shape and in degree of slope There was little difference in the growth period of the same batch of broth from day to day, while the shape and position of the standard curve was remarkably uniform

Samples yielding opacities indicating the presence of 0 2 unit or less per ml are discounted owing to the occasional stimulant action of penicillin at this level (Miller *et al*, 1945) Results falling in the zone of 0 8 unit and above are repeated at a higher dilution owing to the loss of accuracy which tends to occur with flattening of the curve in this range

Emphasis must be placed on the use of an efficient water bath, because slight variations in temperature in different parts of the bath reduce the reliability of results considerably

SUMMARY

A turbidimetric test for penicillin assay of reasonable accuracy is described The method has proved suitable for the multiple assays required in production plant control

I wish to acknowledge the technical assistance of Sick Berth Petty Officer C. A. Reading and the staff of the turbidimetric assay laboratory, R.N. Medical School.

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HYPERPIESIS WITH ATHEROMATOUS OBSTRUCTION OF THE RENAL ARTERIES

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(PLATE XCV)

A case of high blood pressure occurring in a young man with advanced atheroma of the main renal arteries at their site of origin from the aorta is described in the following paper. The complete obliteration of the renal arteries by atheroma and thrombosis is thought to have led to the high blood pressure which caused his death. The kidneys appear to have derived their blood supply from vessels entering the renal cortex from the capsule, and from small vessels which had re-canalised the renal arteries. The remarkably slight histological evidence of renal damage resembles the findings in experimental animals after partial obstruction of the renal blood flow (Goldblatt *et al.,* 1934; Wilson and Byrom, 1939, 1941).

Case report

C. C., aged 29 years, was first admitted to Sutton Emergency Hospital in a severe attack of asthma on 1.10.43. Until one week before admission he had been symptom-free and working as a labourer. During this week he had had three attacks of acute paroxysmal dyspnœa.

On examination he was found to have a developmental abnormality of the left arm, a rudimentary hand arising directly from the region of the elbow. There was also a coloboma of the right eye. His heart was enlarged to the left, the apex beat being 4 in. from the mid-line in the sixth space. The aortic second sound was loud but no pulsation could be detected in the right radial or in either brachial artery. In the popliteal artery the blood pressure was 230/135. A trace of albumin was present in the urine.

X-ray examination of the heart showed considerable left-sided enlargement, with pulmonary congestion. An intravenous pyelogram was normal. Blood urea was 45 mg. per 100 c.c., standard urea clearance 48·5 per cent. of normal (11.10.43). No casts or red cells were found in the urine on repeated examination. During an attack of paroxysmal dyspnœa the systolic blood pressure

in the leg had risen to over 300 mm., while the diastolic was 150 mm. The circulation rate (arm to tongue) was 20 seconds between attacks, but rose to 70 seconds during attacks of dyspnœa. His symptoms improved somewhat with rest and the blood pressure in the leg fell to 200/110, but the heart continued to increase in size, the apex beat being 5 in. from the mid-line when he was discharged late in October. We are indebted to Dr O. F. Garai for these valuable observations.

While at home he was able to do very little; he became œdematous and was unable to sleep at night. He was admitted to King's College Hospital on 27.3.44. He was now dyspnœic at rest and slightly cyanosed, the blood pressure in the leg being 230/150. The cardiac apex was in the sixth space in the anterior axillary line and gallop rhythm was audible. There were râles at the left base, ascites and considerable œdema of the legs. The optic fundi were normal. A persistent trace of albumin was present in the urine but no red cells or casts were observed. During sleep Choyne-Stokes respiration was present. Attacks of dyspnœa due to acute pulmonary œdema occurred from time to time.

An electrocardiogram (1.4.44) showed unaccountable right ventricular preponderance. The Wassermann reaction was negative, hæmoglobin 92 per cent. (Haldane), blood urea 21 mg. per 100 o.c., standard clearance 74 per cent. of average normal (5.4.44).

The œdema, which was at first controlled with mersalyl, became more severe, and he complained of considerable pain in the legs. His condition deteriorated steadily and he died on 26.5.44.

Post-mortem examination

Serous effusions were present in both pleural cavities and the bases of the lungs were œdematous and congested. There were also ascites and a small pericardial effusion.

The most noticeable changes were found in the aorta and its main branches, all of which were hypoplastic. The whole of the aorta showed extensive patchy atheromatous degeneration and thickening, the orifices of its branches being particularly affected. The lesions consisted of slightly raised yellowish plaques of typical early atheroma, together with thicker and more elevated areas of greyish-white colour. These, when cut across, were seen to contain soft atheromatous material lying between the thickened intima and the media. Histological examination confirmed their atheromatous nature. There was thickening of the intima, with some hyaline degeneration, and the elastic lamina was frayed and split. In the more severe lesions degenerative changes were present also in the media, which in places showed almost complete disruption of the muscular and elastic tissue. In the same areas there was also a little lymphocytic infiltration of the adventitia. No calcification or ulceration was seen.

Both subclavian and common carotid arteries were small in diameter and all appeared grossly obstructed a short distance from their origin. The absence of pulsation in the right radial and in the brachial arteries was thus explained. Sections of the right subclavian artery at the point of obstruction showed considerable atheromatous thickening of the intima, with some hyaline degeneration. There was also thinning and destruction of the media and marked fraying and duplication of the internal elastic lamina. The original lumen had been completely obliterated, though a number of young capillaries were present in the intima and media. A similar appearance was seen in the right common carotid artery, which also contained the remains of an old thrombus undergoing organisation and recanalisation (fig. 1).

The coronary, cœliac and mesenteric vessels were all considerably narrowed near the aorta and sections showed typical atheromatous changes (fig. 2).

These arteries were only partly obstructed. Both renal arteries, however, were completely obstructed for the first few mm. of their course and their orifices would not admit a probe. The right renal artery was examined histologically and the original lumen was not apparent (fig. 3). Two or three small capillaries were seen in the thickened and distorted intima. The distal portion of both arteries appeared normal and there was no evidence of atheroma histologically. The basilar and middle cerebral arteries showed early atheromatous thickening.

The heart was considerably enlarged, weighing 560 g. All the chambers were dilated, as were also the mitral and tricuspid valves. The left ventricle was hypertrophied except anteriorly, where a large area of fibrosis had resulted in thinning of the wall. (Byrom and Wilson comment on the almost invariable presence of myocardial fibrosis in hypertensive rats.) Both coronary arteries were much narrowed by atheromatous change for the first 10-15 mm. of their course, though after that they appeared normal.

The kidneys showed little abnormality on naked-eye examination except for slight scarring of the cortex. The capsules stripped readily and the capsular arterial supply was observed to be rather freer than usual. Histologically, a small number of glomeruli showed thickening of the basement membrane of Bowman's capsule and a little peri-glomerular fibrosis. Occasional glomeruli were shrunken and a very few were completely hyalinised. There was a little patchy fibrosis of the cortex (fig. 4). No hypertrophic or degenerative changes were seen in the renal arterioles. The splenic arterioles were thickened and showed varying degrees of hyaline degeneration. No abnormal changes were found in the vessels of the liver, lungs, suprarenals or stomach.

The liver showed fatty degeneration and marked congestion around the hepatic veins. The spleen and lungs also showed great vascular congestion, with œdema, and small broncho-pneumonic areas were present in the lungs.

DISCUSSION

In this case the main points to be noticed are the youth of the patient, the presence of several congenital abnormalities including hypoplasia of the vascular system, and the severe vascular changes affecting the renal arteries with only slight evidence of renal damage.

Blackman (1939) published an account of the pathology of the renal arteries in "essential" hypertension and described changes ranging from the presence of atheromatous plaques to almost complete obliteration of one or both renal arteries in 43 out of 50 subjects examined at autopsy. In 28 of these cases considerable pathological change was found in the affected kidneys. Arteriosclerotic lesions were found in the intra-renal arteries of all the cases, many of which showed arteriolar necrosis. Richardson (1943) found athero-sclerotic plaques in one or both renal arteries of 25 out of 32 cases of essential hyperpiesia examined at autopsy. In cases where the vascular lesion was unilateral, there was no difference in the appearance of the two kidneys, both of which showed considerable evidence of disease. The two cases with the most marked narrowing of the renal arteries also showed the most advanced renal damage, but changes were not detected in the kidneys of two patients in spite of bilateral arterial stenosis. In rats in which hypertension has been caused by clamping one renal artery, with partial obstruction of blood flow, widespread vascular changes are found in the un-clamped kidney and in other organs but are absent from the vessels of the clamped kidney (Wilson and Byrom, 1939, 1941). This discrepancy between the findings in animals and in man is probably partly due to the much longer time which elapses between the onset of hypertension and the fatal termination in human cases as compared with the comparatively short time (6-41 weeks in Wilson and Byrom's rats) in experimental animals. Differences in the degree and speed of development of the vascular obstruction, rapid in the experimental

HYPERPIESIS IN RENAL ARTERIAL ATHEROMA
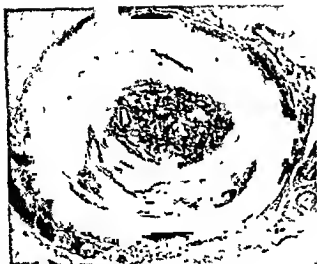
FIG. 1.—Thrombosis of right carotid artery, with complete obliteration of the lumen. Verhoeff's elastic stain and van Gieson. ×10.

FIG. 2.—Atheroma of superior mesenteric artery. H. and E. ×9.

FIG 3.—Obliterated right renal artery. The tear in the centre is an artefact due to the passage of a probe. Verhoeff's elastic stain and van Gieson. ×12.
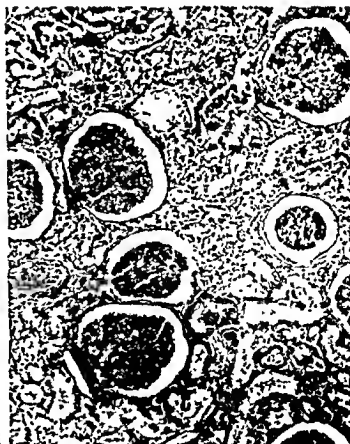
FIG. 4.—Cortex of right kidney, showing patchy fibrosis. One glomerulus (right centre) shows peri-glomerular fibrosis. H. and E. ×75.

animal and relatively gradual in spontaneous disease in man, may also help to account for these discrepancies

It is clearly impossible to determine how long the high blood pressure had been present in our patient before he was first admitted to hospital or how slowly the lesion in the renal arteries had developed the presence of fibrinoid necrosis in the arterioles of the spleen and the relatively short period between the first symptoms in September 1943 and his death in May 1944 suggest that there had been at least a malignant termination, probably with increased blood pressure, and it is probable that this phase did not last more than a year or so The poor condition of the heart muscle consequent on the changes in the coronary arteries was almost certainly an important factor in bringing about a speedy termination It seems probable that there was insufficient time for changes to develop in the kidneys, both of which were in this instance the subject of extreme circulatory disturbances comparable to those induced by the experimental clamping of the renal artery

It is interesting to observe that in spite of the much restricted blood supply to the kidneys, their function was comparatively good and even appeared to improve between the first tests in October 1943, when the blood urea was 45 mg per 100 c c and urea clearance 48 5 per cent of normal, and the later test in April 1944, when the blood urea had fallen to 21 mg per 100 c c and the standard clearance was 74 per cent of normal This suggests that an improved collateral circulation from the capsular vessels and through the re canalised renal arteries themselves had developed during the interval between the tests

The cause of the extensive atheroma in so young a man is not clear The naked eye appearances of the lesions were at first thought to indicate a syphilitic basis, but the Wassermann reaction was negative and the micro scopical appearances were not in any way characteristic of such a pathogenesis The general hypoplasia of the aortic trunk and the presence of other congenital defects suggest that the premature atheromatous change may have been dependent on a congenital or developmental vulnerability of the hypoplastic vessels to the factors which normally cause atheroma in the aged Stenosis of the renal arteries is presumed to have led to the development of hypertension and this in turn may have accentuated the atheromatous change A similar process in the coronary vessels was followed by extensive fibrosis of the ventricular muscle and eventual cardiac failure

SUMMARY

A case of hyperpiesia (B P 230/135—300/150) in a man of 29 with advanced atheroma of the renal arteries is described In addition there was well marked vascular hypoplasia, with other developmental anomalies The histological changes in the kidneys were slight, while the renal function was comparatively good, and even seemed to improve in the six months during which the patient was under observation Comparison is made with the results of experimental vascular obstruction

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576 . 8 . ′077 . 37 : 616 . 46—002 . 2 (lymphogranuloma inguinale)

A COMPLEMENT FIXATION TEST FOR THE DIAGNOSIS OF LYMPHOGRANULOMA INGUINALE

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During 1940-45 a large number of men of several nationalities who had travelled in areas where lymphogranuloma inguinale is prevalent were stationed in this area. This made desirable the elaboration of a laboratory diagnostic test which could be fairly easily performed and which did not demand the use of reagents that are difficult to obtain. Probably the Frei test performed with an antigen of human origin will remain the method of choice, but extracts of lymphogranulomatous lesions in man are not readily obtainable, while similar preparations derived from animal lesions—for example extract of infected mouse brain—give dermal reactions whose interpretation is not easy.

From our experience of the diagnosis of smallpox and the investigation of *the immunity response to influenza we felt that a complement fixation procedure* would probably be the most satisfactory type of serological test to employ for the examination of cases of lymphogranuloma inguinale. It was essential for this that antigens suitable for complement fixation reactions should be prepared.

The possible sources of such antigens are :—

1. Extracts of human lesions. This is probably the best source and was used by Coutts and Ponce (1934-35), but is not readily obtained, and if obtainable it could appropriately be used for the Frei test.

2. Mouse brain passage virus. This we found unsatisfactory in that it was difficult to prepare and was frequently anticomplementary.

3. Culture virus—" lyngranum "—obtained by growth in the yolk sac of the developing chick has been the antigen employed by most workers, the procedure being that described by McKee, Rake and Shaffer (1940), Shaffer, Rake and Grace (1942) and Blair (1944). This was found difficult to prepare in sufficient quantity with the resources at our disposal.

4. Mouse lung passage virus prepared from a lung-adapted strain of the causal agent.

Experience in conducting complement fixation tests with antigens prepared from lung tissue induced us to employ the last of these, and we are indebted to Dr C. H. Andrewes for placing at our disposal the van den Ende lung-adapted strain of the virus.

We are not unmindful of the fact that lymphogranuloma inguinale is but one of a group of diseases due to virus agents which show serological group relationships to one another—lymphogranuloma, psittacosis, " meningopneumonitis of mice ", virus pneumonia of mice and atypical pneumonia of man—and that group reactions might occur. Attention has been drawn to this by Rake, Eaton and Shaffer (1941) and Levine, Holder and Bullowa (1943), while Florman (1945) lays special emphasis upon such group reactions when lyngranum is employed in complement fixation tests. The main source of error from this would be atypical (virus) pneumonia but positive cold agglutination using Moss group IV blood would be obtained with at least some of them.

Again we fully appreciate that lung consolidation in mice can be caused by several virus agents. It was therefore necessary to ensure that all batches of lung antigen reacted well with the serum of a known case of lymphogranuloma, before being used for diagnostic work. Even this is only a partial safeguard, so that in all instances in which a positive diagnostic reaction is obtained it is essential to take into account the clinical manifestations of the case.

Technique

(i) *Antigen.* Mice inoculated intranasally with infected lung extract are killed on the 4th day and the consolidated portions of their lungs dried *in vacuo* from the frozen state. For the actual test 0·1 g. of dried powder in 50 c.c. of saline (= 1 : 500) is used.

(ii) *Complement.* Pooled complement from several guinea-pigs is employed : it is convenient to use this also as a product dried from the frozen state. Preliminary tests of activity are set up in the presence and in the absence of the antigen and it is essential that the antigen is itself not anticomplementary.

(iii) *The test proper.* The patient's serum, inactivated, is diluted 1 : 4, 1 : 8, 1 : 16, 1 : 32 and 1 : 64, and of these dilutions 0·1 c.c. is put into two sets of five tubes; 0·125 c.c. of antigen is then added to one set of five tubes, 0·125 c.c. of normal mouse lung extract to the second set. To all tubes complement containing 2·5 minimal hæmolytic doses in 0·125 volume is finally introduced, the tubes shaken and the mixtures incubated for 1½ hr. at 37° C. After this, 0·125 c.c. of a 3 per cent. sensitised suspension of sheep corpuscles is added and the tubes further incubated in a water-bath for 30 minutes.

A reading can then be taken, but it is convenient to stand the tubes in a cool place overnight so that the intact corpuscles can sediment. It will be noted that the final concentration of patient's serum in the series of tests is 1 : 14, 1 : 28, 1 : 56, 1 : 112 and 1 : 224.

Results

In the first instance tests were made with sera—246 in number—derived from cases known *not* to be infected with lymphogranuloma inguinale. Among these were sera giving both positive and negative Wassermann reactions, also sera giving positive fixation with gonococcal antigen. This series was investigated to ensure that the mouse lung antigen did not react with normal sera, Wassermann-positive sera or the sera of patients containing antibodies to the gonococcus.

Being satisfied that non-specific reactions did not occur with such sera, tests were made with serum from cases of known and suspected lymphogranuloma inguinale. During the period 1st March 1944 to 18th July 1945, fifty-seven specimens from cases in which the history of the patient or his condition suggested the possibility of lymphogranuloma inguinale infection were examined. Of these, 31 gave positive and 22 negative findings. The four additional specimens were from *known* cases of the malady, with typical clinical manifestations and a positive Frei reaction. They were kindly supplied by Drs MacCallum and Bauer to act as positive controls.

As to the degree of reaction obtained :—

(a) In 3 instances fixation occurred only in the first tube = 1 : 4
(b) In 9   ,,   ,,   ,, to the second tube = 1 : 8
(c) In 8   ,,   ,,   ,, to the third tube = 1 : 16
(d) In 5   ,,   ,,   ,, to the fourth tube = 1 : 32
(e) In 6   ,,   ,,   ,, to the fifth tube = 1 : 64

Of the four specimens from known cases, three reacted in a dilution of 1 : 64 and one in 1 : 32.

The following are illustrative examples from each of these.

(a) *Reacting in* 1 : 4 *only.* These were but three and in none of them was the history or clinical condition really suggestive of lymphogranuloma.

(b) *Reacting to* 1 : 8. **Example 1.** Examined July 1945, female ; ulcers on cervix and enlarged lymph glands. Wassermann negative ; gonococcus fixation positive. Case still under investigation. The case is one of old gonococcal infection and the fresh infection does not have the clinical features

of gonorrhœa. **Example 2.** Examined June 1944, male; gonorrhœa 1933, inguinal bubo lasting two months 1935. Wassermann negative; gonococcus fixation test positive.

In none of the 9 cases in this group in which the sera reacted to 1 : 8 only was the clinical condition such as to indicate lymphogranuloma inguinale, but the two cases selected as examples suggest that this degree of response may be suspicious, since the first case may yet develop a more marked reaction and the second may be residual from 1935.

(c) *Reacting to* 1 : 16. **Example 1.** Examined January 1945, male; clinically lymphogranuloma of three years' duration, Wassermann negative; gonococcus complement fixation test not done. **Example 2.** Examined June 1944, male; no evidence of lymphogranuloma inguinale infection, at present suffering from gonorrhœa, examination for gonococci positive; gonococcus complement fixation test positive.

Example no. 2 of this category is important as it indicates an experimental error and serves to stress the need for correlation of clinical and laboratory findings in interpreting the result of this test. This is true of all such tests, none of which is accurate to 100 per cent.

(d) *Reacting to* 1 : 32. **Example 1.** Examined June 1945, male; diagnosed clinically as possibly pestis minor. Cultures from gland yielded no growth, aerobically or anaerobically, on a variety of media. Gland broke down and healed slowly under sulphonamide treatment. Wassermann negative; gonococcus fixation test not done; no evidence or history of gonorrhœa. **Example 2.** Examined April 1944, female, as a known contact of a case of lymphogranuloma inguinale. Wassermann reaction negative; examination for gonococcal infection negative.

(e) *Reacting to* 1 : 64. In all instances in which a reaction of this nature was obtained there was evidence of either present or past infection with lymphogranuloma inguinale.

(f) Among those reacting to 1 : 8 there was a case of some interest—that of an individual who had a non-specific purulent urethritis. All examinations for gonococcal infection were negative, as was also the Wassermann reaction.

Conclusions

The number of cases examined is admittedly small but the findings indicate that the procedure is worthy of more extended trial. Owing to the small number of specimens available for study the experimental error of the method could not be assessed. On the other hand its application to suspicious cases has revealed a few that would otherwise have escaped detection. These cases are probably not important clinically if males, but may not be devoid of significance if females.

The reaction is diagnostic when fixation occurs in 1 : 32 and 1 : 64 dilutions of the patient's serum, is suspicious when positive with 1 : 16, but when positive only with higher concentrations one would hesitate to express an opinion unless the clinical findings were characteristic.

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SPONTANEOUS ABDOMINAL FAT NECROSIS IN A MOUSE

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(PLATE XCVI)

Fat necrosis as seen in the subperitoneal fat in man has usually been attributed to injury or disease of the first part of the duodenum, of the pancreas or its duct, or of the biliary apparatus In dogs, cats, rabbits, guinea pigs and rats the same sort of abdominal fat necrosis has been caused by the intra peritoneal injection of fresh sterile pancreatic juice (Frugoni and Stradiotti, 1910) Contamination with bile or bacteria, at one time thought essential for the production of the lesion, was shown by this experiment to be unnecessary. Widespread subperitoneal fat necrosis of this kind is now generally attributed to the diffusion of pancreatic lipase, which splits fat into glycerol and fatty acids The glycerol is dissolved and absorbed and the fatty acids combine with various bases to form soaps, meanwhile the affected tissue becomes necrosed and infiltration of the surrounding tissue with leucocytes ensues

The following example of fat necrosis, identical in character with that seen occasionally in man, occurred without known cause in a stock breeding mouse This mouse was 280 days old when killed and had borne three litters, the last of which was delivered 40 days before the animal was killed , the young had been successfully nursed by her On the day of her death Mr Ewers, the technician in charge, found that, though the mouse seemed perfectly well, she had two large intraperitoneal swellings These were strikingly hard, freely movable and apparently painless, and might have been thought to correspond with the uterine horns No diagnosis was made and the mouse was killed with chloroform At the post mortem examination the large symmetrical swellings were found to be the genital omenta, which were bulky, hard and almost chalky white, as was all the rest of the subperitoneal fat, including that around the kidneys and pancreas, in the intestinal mesenteries and elsewhere Neither free fluid nor haemorrhages were present in the abdomen In every other respect the mouse was healthy, and no fat necrosis was present except in the subperitoneal tissue Macroscopically no lesion of pancreas, duodenum, liver or biliary apparatus was discovered

In mice the pancreas is a widely distributed organ, and in our microscope sections it was obvious that the pancreatic cells in some parts of the organ failed to take up a normal amount of stain It may be that these poorly coloured cells represented a primary disease of the pancreas, but it seems more probable that the cells had been injured secondarily by the toxic action of free fatty acids, liberated through the action of pancreatic lipase, on the adjacent fat with which the cells were in contact

Sections were made of the subperitoneal adipose tissue in various regions: they all showed the typical picture of fat necrosis. The affected areas were but feebly stained and showed infiltration of the surrounding tissue with leucocytes (figs. 1 and 2).

The mouse has been preserved in the museum of the Royal College of Surgeons of England.

Summary

A case of generalised subperitoneal fat necrosis without obvious cause in a mouse which had littered 40 days previously is described.

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616.155.34

THE NATURE OF NEUTROPHILIC GRANULATION

G. DISCOMBE

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If smears of bone-marrow are stained for 25-30 mins. in Leishman's stain (B.D.H.) buffered with a phosphate buffer containing 2 molar proportions of dihydrogen to 1 of monohydrogen phosphate, the stain merely rinsed off without differentiating and the smear blotted, then the pro-myelocytes and a few myeloblasts with nucleoli show in their cytoplasm a pale-staining area around which the specific granules first appear. Such cells appear to correspond with the myelocytes "A" and the less granular with the myelocytes "B" of Sabin, and it would be a reasonable hypothesis that the pale-staining area contains a Golgi apparatus.

Attempts to demonstrate such an apparatus by Baker's (1944-45) calcium-formol-Sudan black technique failed, but it was found that the specific granules both of neutrophils and of eosinophils were coloured by the Sudan black and hence consisted largely of lipoid. It was also noted that the size, appearance and distribution of these granules were indistinguishable from those seen in a good oxidase preparation (see also McManus, 1945).

Search showed that an extensive literature clearly indicating the lipoid nature of neutrophilic granules existed and was reviewed by Schrt (1927) and Lison (1936). It appears highly probable that the localisation of dye in oxidase-positive granules is secondary. Enzymes present in cytoplasm or nucleus catalyse oxidation of the reagent, and as the product of oxidation (indophenol or benzidine blue) is more soluble in lipoids than in water, the lipoid granules extract the blue from the cytoplasm. As Lison points out, it is possible that the oxidase reaction depends, not on the presence or absence of the oxidase, but on the presence in the cell of lipoid in a form which can dissolve and thus retain the oxidation product.

The very great difference between the appearance of neutrophilic granules stained by Leishman's stain and those coloured by Sudan black appears to be an optifact, one of the azurs (probably Bernthsen's violet) being adsorbed on the surface of the lipoid granule, and light from the condenser being focussed by the granule to form a disc of much less diameter than the granule itself. It has been found that short fixation in Leishman's stain does not extract the lipoid appreciably. Incidentally, the term neutrophil seems to be a misnomer,

PLATE XCVI

SPONTANEOUS FAT NECROSIS IN A MOUSE
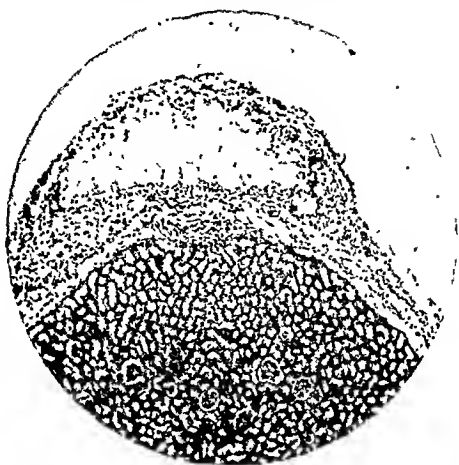
FIG 1.—Section through perirenal fat showing a focus of unstained (necrotic) adipose tissue surrounded by leucocytes. × 45.

FIG. 2.—Section through genital omentum presenting the same features as fig. 1. × 45.

for staining with a simple aqueous solution of Bernthsen's violet, a basic dye containing no eosin, demonstrated the granules well

It is a matter for regret that this very extensive work has not reached the standard hæmatological reference books, though similar information on the eosinophils has done so, it would have spared many workers much fruitless labour

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576 . 851 . 57 (*Cl welchii*) : 576 . 8 . 097 . 36

THE FIXATION OF *CL WELCHII* TOXIN BY SKIN CELLS

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The development of local skin necrosis at the site of intradermal injection of toxic filtrates of cultures of *Cl welchii* was described by Klose (1916) On the basis of this observation he devised a method for estimating the residual toxic activity of toxin antitoxin mixtures which has since been used frequently for the titration of preparations of this antitoxin Klose gave no account of the sequence of changes which culminated in the necrosis, but the appearances of the lesions at various stages of their development have since been briefly described by Glenny *et al* (1931) The present experiments were made to determine how soon after its injection intradermally, the necrotising toxin becomes so fixed to the tissue cells that it can no longer be neutralised by antitoxin Clearly, the rate of fixation of a toxin is a factor which affects importantly the readiness with which it can be countered therapeutically with antitoxin

The toxin used in these experiments was prepared from a filtrate of a six hour culture of *Cl welchii* type A (S 107) in a serum peptone medium by precipitation with ammonium sulphate its LD_{50} dose, as determined by the median death time method of Ipsen (1941), was 0 4 mg for mice All the toxin solutions injected were freshly prepared from dried material on the day they were used the quantity usually employed was 1 mg in 0 2 c c of saline, though smaller doses were sometimes used The injections were made intra dermally in the flanks of albino guinea pigs The inoculation of the toxin was followed at intervals ranging from 30 seconds to 2 hours by injections of specific antitoxin (0 1 c c of a solution of antitoxic globulins containing 200 units per c c) through the same needle hole and in the same direction Since the areas of necrosis were often large and tended to spread rather widely in some animals, only six injection sites were used in each guinea pig in four of these sites the toxin was followed by antitoxin, and in two, placed diagonally on opposite sides, it was left unneutralised to produce control reactions

The sequence of changes observed in the control sites closely resembled those described by Glenny *et al* Those at the sites injected later with antitoxin varied

from no reaction when the interval was 30 seconds or 10 minutes, through a slight erythema when it was 20 minutes, to a full necrosis, sometimes slightly smaller than but usually indistinguishable from those at the control sites, when the interval was 30 minutes or longer. The findings in seven representative animals are set out in the following table.

TABLE

Skin reactions after injections of toxin followed by antitoxin

Guinea-pig no.	Interval between injections					
	30 secs.	10 mins.	20 mins.	30 mins.	Controls	
1	—	tr	±	+++	+++	+++
2	—	—	±	++	+++	+++
3	—	—	—	++	...	+++
4	—	—	±	++	++	+++
5	—	tr	±	++	++	+++
6	—	—	±	+++	++	+++
7	—	tr	±	+++	++	+++

tr = slight erythema
± = erythema 3-4 mm. diameter
++ and +++ = well defined areas of necrosis

Cl. welchii type A filtrates usually contain two toxins, α and θ, both of which are capable of producing skin necrosis, though the action of the θ toxin in this respect is much weaker than that of the α toxin (Oakley, 1943). Two pieces of evidence support the view that the necrosis observed in the present experiments was caused by the α toxin, or lecithinase, present in this reconstituted fraction of the toxic filtrate. The first is that the necrotising action of the solution injected was not recognisably diminished when its θ toxin component had been removed by absorption with sheep red cells at 0° C. by the method described by van Heyningen (1941). The second is that if the solution injected were brought into contact with purified lecithin for one hour at room temperature (Zamecnik *et al.*, 1945) and subsequently filtered until clear, it lost all its former necrotising power. The skin necrosis observed seems thus to have been due to the α toxin.

Although the local infiltration with antitoxin of the previously intoxicated site after intervals of up to 20 minutes practically annuls the reaction which develops in an almost uninterrupted manner if the intervals are longer, it does not necessarily follow that the fixation of the toxin to the cells takes place 20-30 minutes after its intradermal injection. Should there be some delay in the union of toxin and antitoxin, it might happen that for a period of uncertain length the two might coexist uncombined in the tissue spaces. Although this possibility has not been eliminated in the present experiments, it seems intrinsically unlikely from our general knowledge of the rates of combination of homologous antigens and antibodies. Moreover, in this particular instance it is known that the speed of combination of *Cl. welchii* α toxin with its antitoxin is very rapid from the fact that the turbidification of a lecitho-vitellin solution by this toxin is abruptly terminated by the addition of an excess of its antitoxin. From the immediate cessation of the lecithinase activity upon the addition of antitoxin, it seems fair to conclude that a comparably rapid neutralisation of the toxin takes place in the tissue spaces in these experiments and that the above period of 20-30 minutes probably indicates correctly the time required for the fixation of the toxin to the susceptible skin cells.

The rapidity of fixation of the α toxin of *Cl. welchii* contrasts sharply with

the comparative slowness with which small doses of diphtheria toxin became similarly fixed after intradermal injection (Wright and Clark, 1944). This difference too is reflected in the differences in the death times following the intravenous injection of large quantities of the two toxins: the minimum death time with diphtheria toxin is about nine hours, while that for *Cl. welchii* toxin is only a few minutes. The rapid fixation of this latter toxin is probably associated with the prevalence throughout the body of the substrate lecithin upon which the toxin acts: it is possible that the rate of fixation of other toxins may be found to depend upon the availability and accessibility of their particular substrates. In any case, differing rates of fixation of toxins must have a significant bearing on the likely value of specific antitoxin therapy following infection.

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ANTHRACOSIS OF THE LIVER

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(PLATE XCVII)

From time to time, in cases of marked anthracosis of the lungs with or without silicosis, metastatic deposits of carbon pigment are found in the spleen, liver, bone marrow and kidneys. These deposits are usually small, only occasionally marked, and as a rule are blood-borne, although spread by lymphatic channels may also occur.

The earliest recorded instance of "blood anthracosis" is that of Soyka in 1878 (quoted by Weigert, 1883), in which carbon pigment was found in the spleen, liver and kidneys of a man of 70 years, suffering from anthracosis of the lungs. Soyka formed the opinion that the carbon had passed through the lymph glands and thoracic duct into the blood stream. Weigert found deposits of carbon in the spleen and liver not infrequently in old subjects in Leipzig and he described the direct entry of carbon from heavily dust-laden bronchial lymphatic glands into the blood stream.

Ohkubo (1908) made like observations in a series of 44 cases of anthracosis and emphysema in elderly people (6th-8th decade), and emphasised the infiltration by carbon of the walls of small veins throughout the lung substance and the subsequent dissemination in the blood stream.

We have now seen ten examples in elderly subjects (65-80) of cicatricial anthracotic lesions of the large pulmonary veins and arteries in the hilum of the lung in association with deeply pigmented and adherent hilar glands. In nine of these ten cases, however, there was only moderate anthracosis of the pulmonary parenchyma, emphysema and silicosis were absent and there were no metastatic pigment deposits of note. The 10th case is here reported on account of the association of gross anthracosis of the liver with characteristic pigmented cicatricial ulceration of the large pulmonary veins in relation to subjacent anthracotic and silicotic lymph nodes.

Case report (A. 2808)

J. D., a Lanarkshire miner aged 81, died 18 hours after excision of an epitheliomatous growth of the lip. Post-mortem examination showed very severe silicosis and anthracosis with cavitation, and anthracosis of the liver.

Liver. This was of normal weight and of a general greyish colour due to marked pigment infiltration. On section the lobular pattern was rendered unusually distinct by the black deposits of carbon in the portal tracts, while the intervening parenchyma was yellowish grey. In the major portal tracts the pigment was more distinctly seen in the lymphatic channels accompanying the larger portal veins, hepatic arteries and bile ducts (fig. 1). On the capsular aspect the injection of subcapsular lymphatics with carbon was most distinct.

Histologically the pigment is identified both in the lymphatics of the portal tracts and in the Kupffer cells, many of which are engorged with carbon (fig. 2). The distribution is quite irregular, differing in this respect from malarial pigment, and occasionally it occurs in larger deposits. None, however, is contained within the liver cells. There is no distinct increase of fibrous tissue in the portal tracts.

Lungs. Both pleural cavities were almost completely obliterated by old, dense, fibrous adhesions. The lungs exhibited an extreme degree of anthracosis, being uniformly black in colour. In each upper lobe irregular fibrosis and cavitation were present. The cavities contained very soft black material of creamy consistency and had walls of cartilaginous hardness. There was a general increase of fibrous tissue throughout both lungs and firmer nodules were frequently felt. The presence of silicosis is confirmed histologically, but there is no evidence of tuberculosis. The bronchi show chronic catarrhal changes.

Bronchial lymphatic glands. These were small, hard and gritty, densely black and firmly adherent to adjacent structures, especially the larger pulmonary veins. The pulmonary arteries were relatively healthy and showed little atheromatous change.

Pulmonary veins. The larger pulmonary veins showed foci of pigmentation and scarring, which in a few instances had proceeded to ulceration. The earliest form of the lesion appeared as a grey patch on a smooth intima ; in later stages the pigmentation was greater and the intima varied from translucent and slightly wrinkled to opaque and scar-like. With the latter there was narrowing of the lumen. Occasionally, instead of indrawn radiate scarring, a depressed black plaque surrounded by a grey scarred border was found. The depressed portion was uneven, but otherwise, for the most part, smooth and light-reflecting, although it felt rough and gritty. Three frankly ulcerated areas were noted in the large veins in each lung. These appeared as deeply pigmented patches, with marginal radiate puckering indicative of the cicatricial nature of the lesion ; the surface was only smooth in part, the remainder being ulcerated (fig. 3). The subjacent lung tissue was densely fibrous.

Histologically, sections of the veins, including the subjacent lymph glands, show the latter in a state of advanced anthraco-silicosis and completely fused with the wall of the vessel. In the earlier lesions, where the intima appears

ANTHRACOSIS OF LIVER

Fig 1 —On the cut surface of the liver, accumulations of carbon pigment are seen in both minor and major portal tracts The parenchyma is greyish, due to fatty change plus the presence of carbon pigment in the Kupffer cells throughout the lobules   ×8

Fig 2 —Carbon pigment is seen in many of the Kupffer cells as well as in the lymphatics of the portal tract Hæmalum and eosin   ×150

Fig 3 —A deeply pigmented cicatricial patch is shown in a major branch of a pulmonary vein The upper and lower parts of the patch are ulcerated , the middle portion is smooth but depressed The vein wall, being fairly translucent, appears diffusely grey owing to the intensely black subjacent lung substance   ×2 5
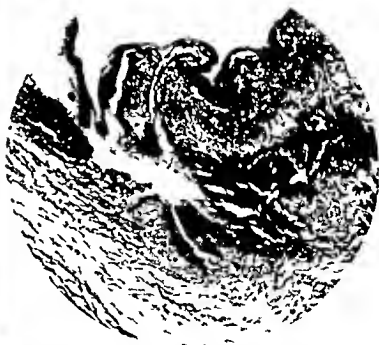
Fig 4 —Section of an anthracotic scar similar to that shown in fig 3 In the right half of the field the vein wall is infiltrated with carbon pigment On the left there is ulceration with destruction, in one part, of the entire thickness of the wall and discharge of carbon from the subjacent accumulation (Just outside the vein at this point there was an anthraco silicotic nodule in an adherent lymph gland) Weigert's elastic tissue stain and eosin   ×8

greyish to the naked-eye, the wall of the vessel is only partially penetrated by pigment ; there remains a thin unpigmented layer which has either not yet been penetrated by the carbon or which has later grown over the deeper layers. In the darker parts the pigment reaches the lumen of the vessel. The advanced lesions show at the periphery of the darkened area much undermining and infiltration of the adventitia by carbon. As the centre is approached more and more of the media and finally the intima become impregnated. At the ulcerated edge free discharge of carbon into the lumen has occurred (fig. 4). The base of such a lesion is densely fibrosed, being part of the periphery of the anthraco-silicotic nodule which has ruptured into the vessel. The small vessels show varying degrees of carbon infiltration. In many instances, more especially in the veins, the infiltration affects the entire circumference and in places the entire thickness of the wall, so that there is much carbon in the intima but probably less getting into the lumen. As in silicosis, obliterative changes, even up to occasional complete occlusion of the lumen of the small vessels, is present.

Spleen. Small amounts of carbon are present in the phagocytic cells of the pulp and in the perivascular lymphatics of the arterioles in the Malpighian bodies.

Bone marrow. No pigment is seen in the portions of rib and femoral bone marrow examined.

Discussion

While carbon may reach the liver and even the spleen via the lymphatics from surcharged pulmonary lymphatic glands, the large amount sometimes found in these organs, as well as its occasional presence in the bone marrow and kidney, suggests that dissemination via the blood stream is the rule. The appearances in the lungs in the present case are in agreement with the findings of Ohkubo and of Weigert, namely infiltration of the walls of the pulmonary veins, small and large, by carbon, with direct escape of the pigment into the circulation.

In the dust diseases the lymphatic glands of the lungs are often fused with the walls of the larger pulmonary vessels. In exceptional instances they are firmly fused even to the walls of the vena cava, or vena azygos. The extent of the fusion varies ; it may affect one side of the vessel only, or, rarely, surround it completely. On opening the affected vessels, the points of fusion correspond to lesions involving most or all of the thickness of their walls. As regards the changes occurring at these points, accumulations of carbon and silica particles within the lymphatic glands set up an inflammatory reaction which results in the destruction, partial or complete, of the lymphoid elements. The formation of a cellular granulation tissue involves the gland, its capsule and the vessel wall, and the destructive and reparative processes, each in varying degree, give rise to the different appearances, early and late. The absence of thrombi in the small ulcerated lesions is probably explained by the too rapid blood-flow in these large vessels.

That such venous lesions will always be found in cases of anthracosis of the spleen and liver cannot be stated with certainty, since, while these form ports for a limited discharge of carbon—limited by reason of cicatrisation—the condition may be so far advanced that the stage of ultimate cicatrisation has been reached, obliterated branches alone remaining. However, having regard to the fact that metastatic deposits of carbon in the liver, spleen and bone marrow may be minimal or even absent in cases where large pigmented cicatrices or ulcers occur in the major pulmonary veins and arteries (as in 9 of our cases), it would appear that the visceral carbon deposits are largely occasioned by the extent and degree of the carbon infiltration of the walls of the small veins in the lung substance.

In conclusion, having regard to the part played by silica in the genesis of

the lesions of the larger pulmonary veins, it is interesting to remark the absence
in this case of metastatic silicotic deposits in the liver and spleen. In general,
these are rare in comparison with anthracotic deposits. Thus as regards the
liver we know of only one example in the literature, that of Welch (1891),
who under the title of *cirrhosis hepatis anthracotica* described a peculiar nodular
form of widespread cirrhosis " dependent upon the presence of coal-pigment ".
There is no doubt from Welch's description of the rounded, pigmented, relatively
acellular sclerotic nodules that they were frankly silicotic foci. Silicotic deposits
in the spleen are also rare (Edinger, 1932 ; Belt, 1939).

Summary

In a case of marked pulmonary anthracosis and silicosis, ulcerative lesions
of the larger pulmonary veins were present, allowing the escape of carbon into
the circulation, and the liver showed a remarkable degree of anthracosis in
consequence. The essential features are described and illustrated. No metastatic
silicotic deposits were encountered.

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A MOTILE ORGANISM OF THE PLEUROPNEUMONIA GROUP

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(PLATE XCVIII)

Early in 1944 we inoculated a number of mice with material believed to
contain typhus rickettsiæ. In fact, no rickettsiæ were present, but one of the
mice developed circumscribed lung lesions. From pieces of this lung cultivated
in Hartley's broth containing 10 per cent. horse serum, we grew an organism
of the pleuropneumonia group.

Cultural characters

The organism grew satisfactorily at 37° C., either in this horse serum broth
or, rather better, in 5 c.c. of Lovinthal broth to which 1·5 c.c. of horse serum
was freshly added. The supernatant fluid became slightly opalescent and,
3 or 4 days after inoculation, a very small granular deposit appeared at the
bottom of the tubes. Subcultivation was carried out by pipetting some of
this deposit into a fresh tube, and so on, about every 7 days. Minute greyish
colonies, scarcely visible without a lens, appeared after 3 or 4 days' incubation on
10 per cent. horse serum agar (fig. 1). Better growth was obtained on Levinthal's
medium made with 1 per cent. agar and mixed, immediately before pouring,
with horse serum (1·5 c.c. to 5 c.c. of medium). The condensation fluid at the

PLATE XCVIII

Fig. 1.—Appearance of colonies on surface of serum agar : 3 days' growth. × 67.

Figs. 2-6.—These show the appearances of cultures under dark-ground illumination. In figs. 3 and 4 are depicted the forms which best show motility. To the left in fig. 3 is a motile organism which had temporarily come to rest ; it had been moving towards the right of the field. In figs. 2, 5 and 6 the organisms are aggregated into small masses of spheres ; fig. 6 shows in addition the highly refractile appearances which suggest the presence of cholesterol, as described in cultures of the L1 organism by Partridge and Klieneberger (1941). × 1500.

A MOTILE PLEUROPNEUMONIA ORGANISM

bottom of a slant of this soft medium was particularly rich in organisms. Subculture was best effected by cutting out from a Petri dish a square of agar well covered with colonies, inverting it on to fresh medium and pushing it to and fro over the surface (Klieneberger, 1938). No growth occurred in liquid or solid media in the absence of serum.

Microscopical appearances

We have chiefly examined the deposit from broth cultures or from the condensation water of slants by dark-ground illumination, using a high-angle dark-ground illumination (Beck) with N.A. 1·20. The organisms resemble others of the group in showing large numbers of globules of various shapes and sizes (figs. 2 and 6); filaments and granules are relatively scarcer than in cultures of Klieneberger's L1 organism.

We failed in one attempt to filter the organism through a gradocol membrane (A.P.D. 0·66 μ), nor did we succeed in preserving it by drying from the frozen state.

Motility

Cultures, especially primary ones from mouse lung, showed varying numbers of motile organisms. At times there were many of these in every field, at other times they required prolonged search. Strings of globules connected by short filaments could be seen moving steadily across the field, or a globule with a short linear stalk would go forward, the stalk in advance, bending slightly from side to side as if feeling its way forward, or a sphere with a projection on its circumference would constantly rotate in one direction. Where a colony clump of spheres was present one globule might be seen to detach itself and move off, or another might approach, enter the cluster and settle down into immobility. The motility was almost exclusively to be seen where the organisms were in very close relation to the slide; they seemed as though crawling in a single plane and were able to move against or across the stream of a gentle convection current. When they became detached and subject to violent Brownian movement, true motility was no longer evident. Careful study failed to show any evidence that the motility depended on the presence of flagella, and indeed the motion as if along a surface would not fit well with such a view. The mechanism remains therefore obscure; it is probably akin to that of some motile myxobacteria. We observed at times that the stalk preceding a moving sphere showed evidence of a wave of thickening passing along it.

Motility became less the longer cultivation *in vitro* was carried out, though it was detected up to the 6th subculture. Passage through a mouse could be carried out by intranasal inoculation under ether and recovery of the organism from the lungs a few days later. During the earlier subcultures this procedure effectively revived the motility of the strain, but after more subcultures, motility was apparently permanently lost, though growth *in vitro* proceeded without difficulty.

Pathogenicity

Intranasal inoculation of deposits from broth cultures produced lesions which macroscopically and microscopically resembled those described by Edward (1940) as produced by the pleuropneumonia-like organisms which he recovered from normal mice. Circumscribed greyish-red lesions were seen in the lungs, especially at the hila, when mice were killed at intervals from 6 to 23 days after inoculation. Intense perivascular cuffing formed the most striking histological feature, but polymorphs were present as well as mononuclears, and (in contrast to Edward's findings) there was some damage to bronchial and bronchiolar epithelium. Attempted serial passage through mice did not enhance the

pathogenicity of the agent; indeed, lesions usually became less on transfer, and it was not always possible to recover the organism from the lungs. The agent was not pathogenic when inoculated intracerebrally or intraperitoneally.

Comparison with other organisms

The motile organism may prove to be identical with one of Edward's, though ours seems to have grown better on solid media than his. We did not carry out a serological comparison. On one occasion we detected motility in a culture, obtained from a mouse's lungs, of an organism corresponding to L5 (Findlay *et al.*, 1938). This produced " rolling disease " on intracerebral inoculation in mice. On plating-out and picking colonies, however, we satisfied ourselves that this was a mixed culture of L5 and a motile organism. The pure L5-like agent was non-motile, nor was the motile organism pathogenic on intracerebral injection.

Summary

We have recovered from the lungs of mice a pleuropneumonia-like organism showing motility. Flagella have not been detected and the mechanism of the motility is undetermined. We are unaware of any previous record of motility in this group.

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615.778—092.259 (Mus) : 576.852.211

THE USE OF MICE IN THE EXAMINATION OF DRUGS FOR CHEMOTHERAPEUTIC ACTIVITY AGAINST *MYCOBACTERIUM TUBERCULOSIS*

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The published experiments on the chemotherapy of tuberculosis have usually been carried out in guinea-pigs or (less often) in rabbits infected experimentally, or else upon naturally occurring cases in man or animals. The scarcity of such reports in contrast to those dealing with the chemotherapy of streptococcal and pneumococcal infections is an indication of the difficulty of this type of experiment. As usually conducted, each guinea-pig experiment may occupy from 6 to 12 months and involves the consumption of a very considerable quantity of the drug. The desirability of a therapeutic test which would enable a large number of chemical compounds to be evaluated in a short time, with the expenditure of a minimal amount of the drug, was stressed by Nitti and Jouin (1942) and Youmans and McCarter (1945). The following account describes a test which has been found satisfactory from these points of view, and which has enabled the preliminary examination of about 200 compounds to be completed during the last 18 months.

The basis of the new method is the regularity in survival time which is observed when a group of mice is infected intravenously with large doses of *Mycobacterium tuberculosis*. With doses of 1 mg. (moist weight) of the organism, mean survival times ranging between 17 and 27 days have been observed. The data obtained in these laboratories from 21 experiments of this type are set out in table I and graphically in the figure.

TABLE I

Results of infecting mice intravenously with 1 mg. of Mycobacterium tuberculosis, " human " type. Detailed results of 21 experiments. All mice untreated

Expt. no	No. of mice in group	Number of mice dead on day																		Survivors	Mean survival time in days *
		14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31		
18	25		1			2	2	7	5	3	4	1								0	20·6
20	36		1		2	6	6	6	4	5	4			1		1				0	20·3
26	24			3	3	4	8	1	1		1	2								1	21·4
28	24		1	2	3	1	2	5	1	3	2	1	1	1						1	20·6
30	22					1		4	3		2	4			3		1		3	3	24·1
32	23							2	2	2	4	5		2	1	1	3			4	26·7
33	12						2	1	2	2	3	1	3	1						0	23·3
34	23			1		1	2	3	3	1	1	2	4	4	1	2				3	25·8
35	24		3	1	3	2	6	5	3	3	1		1			1	1			0	20·0
36	24				2	3	5	3	1	1		1	1	1						3	22·2
37	24		8	2	3	6	1		1	2	1									0	18·4
38A	23		4	1	12	3	1	1	1											0	17·1
38B	24		12		1	7	1			2	1									0	17·9
38C	23		2	3	7	5	2		2	2	1									0	17·9
41	24				2		1	4	3	3	3	5				1				2	23·5
42	23			1	2	2	2	5	3	2			1		2					0	20·7
43	24			1	3	1	4	7		2				1	3					1	21·8
44	24		1		5	4	7		2	1	1	3	2							0	20·5
45	24		1	1	1	1		3	6	4	1	2	1	2	1					0	21·8
46A	23		1	1	1			1	1	3	1	3	5	2		1	1			4	25·2
46B	23		1				1			1		2	4	4	2		1	1	4	3	26·6
Total	496		14	30	33	42	47	58	55	43	37	23	34	21	10	7	8	5	4	25	21·6

* All survivors taken at 32 days (see page 583)

It will be seen that 95 per cent. of the infected mice died between the 15th and 31st days after inoculation. The lung lesions observed under these circumstances have been described by Schwabacher and Wilson (1936-37) and by Stamatin and Stamatin (1939).

Statistical analysis of the data in table I shows that the standard deviation of the survival time for individual mice varies between 2·1 and 3·0 days. The difference between the mean survival times of control and treated groups (both consisting of approximately 24 mice) which would be required for significance at the 19 to 1 level ($p = 0·05$) under these circumstances varies between 1·3 and 1·8 days.

When the inoculum is reduced below 1 mg. the mean survival time of infected mice soon increases very rapidly. This is shown in table II, where the effects produced by inoculating groups of 24 mice with falling weights of organisms are recorded.

Other experiments have shown this increase of survival time to be due to the rapid development of a measure of resistance by the infected mice. This resistance arises as a result of infection with a very small dose of organisms, and is shown by the marked increase in survival time which such " immunised "

mice show to subsequent reinfection with 1 mg. of organisms. When the interval between a small, protecting infection of 0·01 mg. and the subsequent large inoculum was varied between 4 and 30 days, it was found that a marked degree of resistance was developed when this interval was 16 days or more.
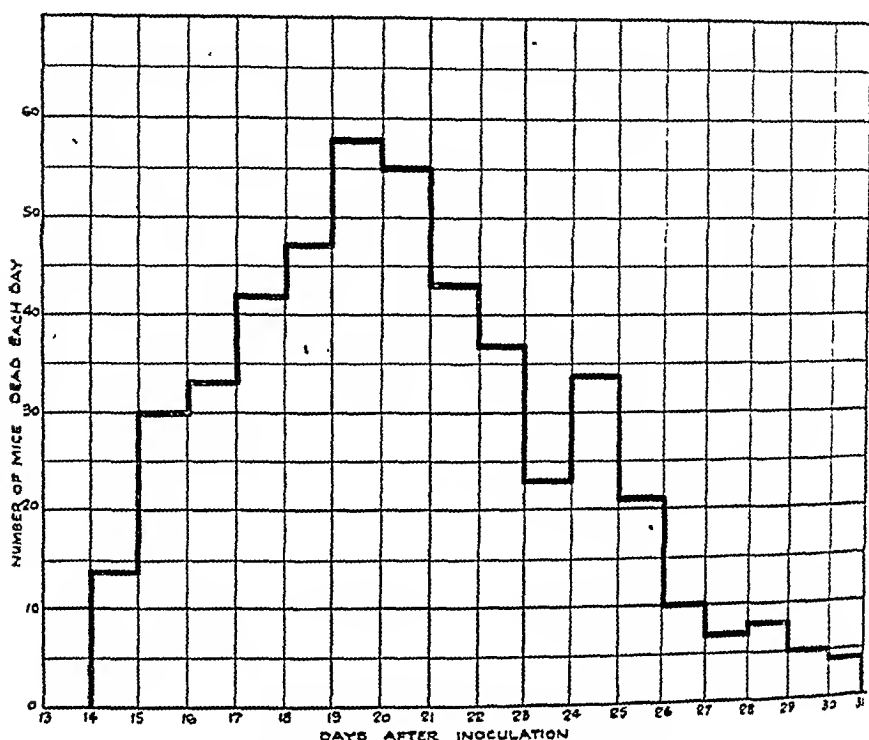
Graphic representation of the data of table I

TABLE II

Deaths resulting from inocula of 1·0, 0·1 *and* 0·01 *mg. of* Mycobacterium tuberculosis *given intravenously to groups of 24 mice*

Group	Inocula	No. of deaths on day																Surviving at 70 days	Mean survival time
		18	19	20	21	22	23	24	25	26	27	28	29	30	31	35	36	45		
A	1·0 mg.	3	3	4	8	1	1		1	2									1	19·1 days
B	0·1 ,,					1	2	2	4	3	3	3		3	3				0	22·6 ,,
C	0·01 ,,															1	1	3	19	>70 ,,

Having ascertained the characteristics of the untreated infection in mice produced by the intravenous injection of 1 mg. of tubercle bacilli, it was of interest to know whether this rapid infection is susceptible to influence by drugs. The conditions which have been adopted as *standard* for this type of experiment and which have led to the successful demonstration of chemotherapeutic action with 4 : 4'-diaminodiphenyl sulphone are described below.

Experimental details of the method

Stock cultures. The strain of organism used, which we have called 905, was selected from 23 freshly isolated human strains, each of which was first examined for mouse virulence by the intravenous injection of 1·0 mg. and 0·1 mg. of growth on Löwenstein's medium. Stock cultures were grown on Löwenstein's medium and kept in the refrigerator. The stock was renewed every three months from organisms dried by the lyophilic method. Batches of 10-20 cultures on Löwenstein's medium in 1-oz. screw-cap bottles, sown from the refrigerated cultures, have always given rapid and abundant growth provided attention was given to the maintenance of an adequate supply of air. Cultures were 17-22 days old when used.

Preparation of suspensions. Suspensions were prepared by milling a weighed amount of growth removed from Löwenstein's medium in bottles containing small steel balls. A total of 15 minutes' milling—at first dry and then with the requisite amount of water—gave suspensions which were satisfactory for the purpose.

Selection and inoculation of mice. In each experiment mice of the same sex 18-24 g. in weight were used. They were randomly distributed into the various groups three at a time until each group consisted of 24 mice. Each group was housed in two large cages each containing 12 mice. All mice were then infected intravenously with 0·1 ml. of the suspension (containing 1 mg. of organisms) prepared as described above. As a further safeguard against changes in technique or in the stability of the suspension, one cage of each group was infected first, followed by all the second cages in order.

Dosing and recording of deaths. Except for special purposes, drugs were administered by mouth, dissolved or suspended in water, using a no. 0 Record needle with a smoothly rounded end as a catheter. In general, doses were given twice daily commencing, with untried compounds, about 1 hour before infection and continuing until the first mouse in the control group died with well developed lung lesions. Any deaths which may have occurred before this were ignored and all deaths occurring after this time were ascribed to the specific infection. Deaths were recorded once daily.

Assessment of results. Where there is 100 per cent. mortality in all groups, comparison of therapeutic effect should be made on the basis of differences between the arithmetic mean survival times of treated and control groups. This may still be done when there are some survivors, these survivors being assumed to die on the day following the period of observation. This is made clear by the example given in table III. In these circumstances the increase in mean survival time required for significance is calculated in the usual way (Fisher, 1944).

When one group contains survivors, a better estimate of the magnitude of any effect produced may be made on the basis of the median survival times, i.e. the times taken for 50 per cent. of the animals in the treated and control groups to die. The median survival time is estimated from a smoothed probit-cumulative distribution curve by the method of Bliss (1937), who also gives a method for assessing the significance of an effect estimated in this way. This alternative method is recommended when the control group contains more than 5 survivors.

The difference in reliability between the two methods is small, the former being the more reliable. The same basis of comparison, mean or median, should be used for all groups in any one experiment.

Demonstration of a chemotherapeutic effect by the method described

The results of a chemotherapeutic experiment carried out under the conditions described are shown in table III. The substances used for treatment were

4 : 4′diaminodiphenyl sulphone and two solubilised derivatives, whose constitutions are as follows.

I.

$$NH_2 \langle \rangle SO_2 \langle \rangle NH_2$$

4 : 4′-diaminodiphenyl sulphone.

II.

$$NaSO_3.CH.NH \langle \rangle SO_2 \langle \rangle NH.CH.SO_3Na$$
$$\underset{(CHOH)_4}{|} \qquad\qquad \underset{(CHOH)_4}{|}$$
$$\underset{CH_2OH}{|} \qquad\qquad \underset{CH_2OH}{|}$$

bis-N-sodium dextrosebisulphite derivative of I.

III.

$$NaSO_3.CH.NH \langle \rangle SO_2 \langle \rangle NH.CH.SO_3Na$$
$$\underset{CH_3}{|} \qquad\qquad \underset{CH_3}{|}$$

bis-N-sodium acetaldehydebisulphite derivative of I.

TABLE III

Results of a chemotherapeutic experiment

Drug used	I			II	III		Untreated controls
Dose in mg./20 g. given twice daily	1	2	3	20	4	6	...
No. of mice in group on day 16 .	24	24	20	23	24	23	24
No. of mice dead on day 17 . .				2		1	2
,, ,, ,, ,, 18 . .	1						4
,, ,, ,, ,, 19 . .	2			1		1	6
,, ,, ,, ,, 20 . .	1	1	1		3	2	3
,, ,, ,, ,, 21 . .	5	3	1	1	4	2	1
,, ,, ,, ,, 22 . .	2	2	2	6	4	4	3
,, ,, ,, ,, 23 . .	3	7	4	5	8	7	1
,, ,, ,, ,, 24 . .	6	3	3	1	2	1	
,, ,, ,, ,, 25 . .		3	2		2		1
,, ,, ,, ,, 26 . .	1	1	1	4			
,, ,, ,, ,, 27 . .	1	1	1	3		1	1
,, ,, ,, ,, 28 . .				1		2	
,, ,, ,, ,, 29 . .		1	1	1			
,, ,, ,, ,, 30 . .		1	1				
Total no. of mice dead from infection with tubercle bacillus	22	23	19	23	24	22	22
No. of survivors at 30 days (assumed to die on 31st day for purposes of calculation)	2	1	1	0	0	1	2
Mean survival time (days) . .	23·0	24·1	23·8	24·0	22·1	23·5	21·9
Increase in mean survival time (days) (a) Observed . .	1·1	2·2	1·9	2·1	0·2	1·6	...
(b) Required for significance (for p = 0·05)	1·8						

It will be seen that the groups treated with 2 mg. and 3 mg. of compound I and with 20 mg. of compound II—which corresponds to Promin (Feldman,

Hinshaw and Moses, 1942)—showed a significant increase in mean survival time when compared with the controls. Compound III (British Patent no. 556,901) produced an increase in survival time (at a dose of 6 mg.) which statistically was not quite significant. The doses of compounds II and III were chosen because previous estimations of the blood levels of free diaminodiphenyl sulphone resulting from their oral administration approximated to those achieved by a dose of 2-3 mg. of compound I. It is felt that these results are almost the best obtainable under these conditions, because repeated doses of 3 mg. of compound I produce very marked hyperexcitability (especially at the beginning of the course) and some mice—four in the present experiment—usually die as a result of the toxic action of the drug.

Although the increases in mean survival time shown in the example quoted are small, they are statistically significant and repeatable, and they serve to demonstrate the practicability of the method. Considerably larger increases in mean survival time have been obtained in other experiments by the use of compounds unrelated to diaminodiphenyl sulphone. It is hoped to report these results in detail in the near future.

Summary

Observations on the response of mice to graded intravenous doses of *Mycobacterium tuberculosis*, " human " type, show that small doses (*e.g.* 0·01 mg.) rapidly induce a condition of high resistance to reinfection with a large dose. A dose of 1 mg. given in this way to mice not previously infected causes the death of 95 per cent. of the animals in approximately 21 days.

This rapidly fatal infection forms a convenient basis for the preliminary assessment of the chemotherapeutic power of drugs. An increase in mean survival time of treated over control animals of 1·2 days is significant at the $p = 0·05$ level. The method of standardisation of manipulative details which makes this practicable is described.

A typical experiment using 4 : 4'-diaminodiphenyl sulphone and two solubilised derivatives demonstrated significant increases in mean survival time of the treated animals as compared with the controls.

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616 . 46—002 . 951 . 3 (Wuchereria)

FILARIAL EPITROCHLEAR GLAND

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(PLATE XCIX)

Clinical summary. The patient, a girl aet. 14, had complained of pain and numbness in the right arm and pain over the back of the right shoulder for about 1 year. There was loss of sensation to cotton wool and pin-prick over the inner aspect of the lower third of the right arm and upper forearm. The palpebral fissure and pupil of the right eye were smaller than those of the left. The following lymphatic glands were palpably enlarged :—the upper and lower jugulars on both sides, the central and pectoral groups of the right axilla and the central group of the left axilla. The right epitrochlear gland was enlarged ($\frac{1}{2}$ in. in diameter), movable and tender. It was excised for microscopical examination. The liver and spleen were not enlarged. The peripheral blood was negative for microfilariæ and the absolute eosinophilic blood count was within normal limits.

Microscopical findings. With the low power, the excised lymphatic gland shows marked follicular and reticulo-endothelial hyperplasia with much eosinophilic infiltration. There is some fibrosis, especially peri-glandular and surrounding dilated lymphatic vessels in which numerous sections of an adult filaria can be seen, cut in various planes (fig. 1). Under the high power, numerous coiled up basophilic microfilariæ can be made out in some of the worm segments, lying free in the interior of the uterus (fig. 2), the strongly eosinophilic double-contoured wall of which stands out prominently. The lymphatic space enclosing the worm contains also some albuminous material, many lymphocytes and an occasional eosinophil cell.

Remarks. The case is interesting in that while an adult worm with microfilariæ *in utero* is demonstrated in the excised epitrochlear gland there is neither a hæmic eosinophilia nor demonstrable microfilariæ in the peripheral blood. Nor is there evidence of the typical allergic response to the parasite in the affected lymph gland, which shows neither necrosis nor giant-cell reaction. There is also a remarkable absence of constitutional symptoms in spite of the widespread lymphadenopathy. It is impossible to say whether the infestation is one of *Wuchereria bancrofti* or *W. malayi*, both of which are found in Ceylon.

611—018 . 46 : 618 . 2

THE BONE-MARROW IN NORMAL PREGNANCY

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(PLATE C)

Reports on the appearance of the bone-marrow in normal pregnancy are few and the views expressed by different observers are not in agreement. Forssell (1939) and Pitts and Packham (1939) found no significant difference between marrow counts in pregnant and non-pregnant women, but Forssell's observations were made chiefly on women in the early months of pregnancy,

FILARIAL EPITROCHLEAR GLAND

Fig 1 —Low power view of epitrochlear lymph gland showing two different portions of the adult filaria, one cut transversely the other obliquely, lying within a dilated lymphatic channel The lymphoid tissue generally is much fibrosed

Fig 2 —High power view of the transverse section of the filaria showing numerous microfilariæ in the interior of the uterus.

and Pitts and Packham aspirated 10 c.c. of material and would thus tend to get a greater dilution with blood.

At the other extreme Daniachij (1936) reported a shift to the left of the granular series, becoming more marked as pregnancy advanced, and an apparent megaloblastic reaction in the marrow, reaching a maximum at the seventh or eighth month of pregnancy. The percentage of cells classed as megaloblasts in his series of 32 patients varied from 0·1 to 1 per cent. He used as his standard of comparison the normal figures obtained by Arinkin. Russo (1937) also classified some cells as megaloblasts in making counts on the bone-marrow of normal pregnancy, but admitted that these were large cells in mitosis whose nature could not readily be determined.

Hansen (1937-38) compared his results with the normal ranges of Segerdahl and Nordensen and deduced that there was a shift to the left of the granular series. He also thought that increased erythroblastic activity was indicated by the occurrence of clusters of macro-normoblasts, but he found no megaloblasts. It should be noted, however, that Arinkin's figures for the normal range differ considerably from those of Segerdahl and Nordensen and if Daniachij and Hansen had reversed their choice of normal figures they might have been led to different conclusions.

Markoff's (1939) studies were purely morphological. He again found no megaloblasts, but he observed an increase in erythropoiesis, with the formation of islands of normo- and macroblasts. This commenced in the second month and reached a maximum in the sixth month of pregnancy. In the white cell series he found giant pro-myelocytes from the second month onwards, and in addition some anisocytosis among the myelo- and metamyelocytes. He also noted an eosinophilia in the marrow after the sixth month and a plasma cell type of reticulum cell reaction beginning as early as the third month and reaching its height from about the sixth month onwards. The value of these observations, which are illustrated by excellent photographs, would be greatly increased if there were some indication of the number of patients investigated.

Personal observations

The following observations were made on nineteen healthy pregnant and puerperal women. None of them showed any anæmia at the time of sternal puncture (the lowest hæmoglobin value was 84 per cent.), and they were all followed throughout pregnancy and remained perfectly fit. Four of the women who had been first seen before the third month of pregnancy had a second sternal puncture shortly before delivery (R. H., J. A., J. R., and E. B. in fig. 1).

On each marrow a differential count was made on 500 cells. For comparison with non-pregnant women of the same age group, counts were made on the marrow from ten healthy volunteers.

In view of the suggestion made by previous observers that an erythroblastic reaction is seen in the marrow in pregnancy, especially in the later months, the percentages of the red cell precursors (pro-erythroblasts and erythroblasts) have been plotted against the duration of pregnancy in weeks (fig. 1). The non-pregnant and puerperal groups are included in the same diagram. No clear relationship is shown by this means. The question has, however, been further investigated by statistical methods.

The t test (Fisher, 1944, p. 120) was used to compare the percentages of red cell precursors in the following pairs of groups :—

    I. Non-pregnant and pregnant women.

    II. Non-pregnant and puerperal women.

    III. Non-pregnant women and women in the last eight weeks of pregnancy.

    IV. Four women (R. H., J. A., J. R. and E. B.) early in pregnancy and the same women late in pregnancy.

The results of I and II have no significance (P between 0·4 and 0·5, and 0·5 and 1·0 respectively). In III, on the other hand, P lies between 0·05 and 0·02. The suggestion that this is significant should, however, be made with reservation, for it must be remembered that many uncontrollable factors enter into the making of marrow smears and differential counts. In such circumstances it would be safer to accept as significant only probability values of 0·01 or under.

The results of IV failed to show any significant change in the percentage of erythroblasts in the marrow of the patients in whom sternal puncture was made both early and late in pregnancy (P between 0·1 and 0·2).

The χ^2 test (Fisher, 1944, p. 85) was used to determine whether there was any shift to the left of the erythroblast series in the latter half of pregnancy. The frequencies of pro- and basophil erythroblasts, and polychromatic and
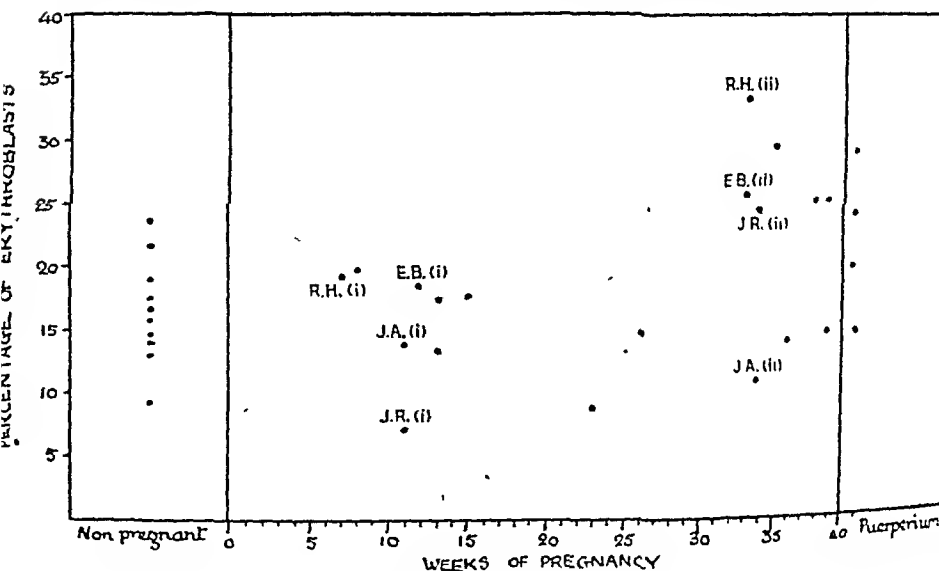
FIG. 1.—Scatter diagram of the percentage of red cell precursors in relation to the duration of pregnancy.

orthochromatic erythroblasts were considered in relation to the first and second half of pregnancy. The results did not indicate any significant change (P > 0·7).

Morphology

In commenting on the morphology of the marrow, it is important to bear in mind the variations which may be encountered in normal non-pregnant women; otherwise undue emphasis may be laid on points which appear to constitute deviations from the normal.

In some of the pregnant, puerperal and non-pregnant series, large enough fragments of marrow were obtained for sections to be prepared. Considerable variation in cellularity was observed in each group, but there appeared to be a tendency towards slight hyperplasia in the late weeks of pregnancy and early days of the puerperium (figs. 2 and 3).

The smears were carefully examined with a view to detecting the changes alleged to be associated with pregnancy and commented on by previous observers. In some cases occasional large erythroblasts showing premature hæmoglobinisation were present and pro-erythroblasts were relatively easily found. None of the nucleated red cells could, however, be classified as megaloblasts.

BONE-MARROW IN PREGNANCY

FIG. 2.—Section of sternal marrow in a non-pregnant woman. ×480.
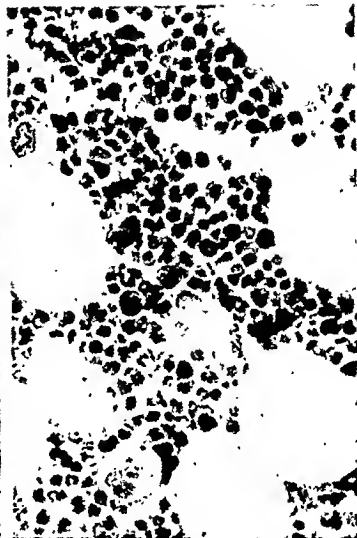
FIG. 3.—Section of sternal marrow in a patient 36 weeks pregnant. ×480.
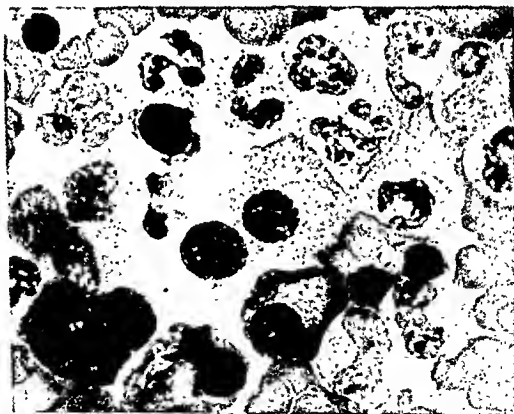
FIG. 4.—Group of reticulum cells of plasma cell type in a patient 35 weeks pregnant. ×1100.

In individual smears erythroblasts occurring in clumps, occasional large pro-myelocytes and groups of plasma cell type reticulum cells were found (fig. 4). These were possibly all more noticeable in the later months of pregnancy and in the early days of the puerperium. They were not, however, constant findings and similar observations could be made on some of the smears from the non-pregnant control group.

It would appear, therefore, that any changes induced in the marrow as the result of pregnancy are not sufficiently striking to justify descriptions of a characteristic "marrow of pregnancy".

Summary

The sternal marrow of nineteen healthy pregnant and puerperal women and ten non-pregnant women has been examined. Statistical analysis of the differential marrow counts fails to give any conclusive evidence that there is an erythroblastic reaction in pregnancy, but examination of sections suggests that there may be slight hyperplasia in the late weeks of pregnancy and early days of the puerperium.

I am grateful to Professor Witts for his advice and to Dr Scott Russell for his help with the statistical analysis. My thanks are also due to the volunteers who were the subjects of this investigation.

The tables of differential marrow counts have been deposited with the Librarian, General Library, British Museum (Natural History), London, S.W.7, and are available for reference there.

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618 . 19—006 . 46—021 . 6 : 547 . 68

THE INDUCTION OF MAMMARY CARCINOMA IN "IF" MICE BY CUTANEOUS AND INTRAPERITONEAL ADMINISTRATION OF METHYLCHOLANTHRENE

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Mammary cancer can be induced by methylcholanthrene in mice belonging to strains which do not develop it naturally. This occurs not only as a local response when methylcholanthrene is injected into the breast, but also as a remote effect when it is injected into the opposite side of the body (Strong and Williams, 1941), or when it is administered intranasally to anaesthetised mice (Orr, 1943). As regards strains which have a natural incidence of breast cancer, Engelbreth-Holm and Lefèvre (1941) and Engelbreth-Holm (1941) found

that 9 : 10-dimethyl-1 : 2-benzanthracene and methylcholanthrene respectively accelerate the appearance of tumours in Little's dilute brown strain. Kirschbaum *et al.* (1944) have since shown that "porcutaneous" applications of methylcholanthrene hasten the onset of mammary cancer in strain Dba mice, the average induction time in breeding females being reduced from 370 to 105 days. The present communication reports the results of the treatment of a cancer-resistant strain of mice with methylcholanthrene by routes other than intranasal.

Experiment I

Thirty-seven virgin female IF mice of the 18th to the 21st generation of inbreeding were used. Methylcholanthrene was applied to the surface of the body as a 0·5 per cent. solution in sweet almond oil. Of this solution, 16 drops (4 on each side of the ventral and dorsal surfaces) were applied, an average dose of 0·25 ml. = 1·25 mg. of methylcholanthrene. The treatment was repeated at fortnightly intervals. In most cases the mice were killed shortly after the appearance of breast tumours.

Mammary carcinoma occurred in 30 of these mice. The earliest tumour was found after 111 days' treatment. Of the mice which did not show mammary carcinoma 3 died before the 111th day, 3 more before the 126th day, and 1 was killed at 118 days when clinical examination revealed tumours in the mammary region which on histological examination proved to be not mammary carcinomata but lymphomata. It may thus be held that mammary carcinoma appeared in every mouse of this group which had received adequate exposure to the carcinogen. The last tumour appeared after 170 days' treatment and the average induction time was 140±2·9 days. The initial age of the mice ranged from 27 to 80 days (mean 45±5·3), and there was no evidence that its magnitude affected the induction time.

An interesting point was the multiplicity of the tumours. As only animals in which attempts at breeding were being made were kept alive, the total number of tumours per mouse is presumably smaller than it would have been had all been allowed to survive. On the other hand, it frequently happened that multiple tumours were first observed simultaneously or almost so : the mouse with 8 tumours, for example, only survived the first finding of a tumour by one week. The number of tumours in individual animals ranged from 1 to 8 (mean 2·9±0·4). The proportionate frequency with which the tumours were distributed in the various mammary regions was much the same as is naturally found in cancer strains.

Pulmonary metastases were found on microscopical examination in 2 animals which survived the appearance of mammary tumours by 41 and 44 days ; only one other mouse was allowed to survive so long. In neither case were the small secondary deposits recognised by the naked eye. Multiple pulmonary "adenomata" were present in 20 instances ; this is a much higher incidence than is ordinarily encountered in this strain. In one case they accompanied secondary deposits of mammary carcinoma. Only in one animal were they present in the absence of mammary carcinoma (the mouse with multiple mammary lymphomata previously mentioned). The mean induction time for mammary cancer in the 19 mice with associated pulmonary adenoma (152±3·8 days) was greater than for the 11 without (139±3·9 days), but it seems unnecessary to attribute this difference to factors other than the more prolonged treatment in the former group.

Attempts were made to determine whether the tendency to develop mammary cancer after this treatment was transmissible to the offspring. Twelve of these mice were mated with brothers, but in no case did conception result. The duration of treatment before mating varied from 86 to 149 days (average 126±7·6 days). Mating took place before the appearance of tumours in 9 instances, immediately after it in 3.

It is somewhat surprising that in this group only one skin tumour occurred, a papilloma of neck which appeared in 183 days, 13 days later than the latest breast tumour It is evident that methylcholanthrene applied in this way induces breast cancer more rapidly than skin cancer, as was the case with intranasal administration The latent period for skin tumours is much longer than when a benzene or acetone solution is applied to the interscapular skin

Multiple lymphomata were found in 4 mice, of which 2 also had mammary carcinoma

Eleven control mice were similarly treated with almond oil alone none developed tumours

Experiment II

In the IF strain skin tumours are induced by hydrocarbons more rapidly than in other mice Reasons were given in a previous paper (Orr, 1943) for discarding the view that the carcinogen may reach the breast tissue from the nipple along the ducts But as the mammary tissue is derived from the epidermis, a further experiment seemed necessary to test the effect of methylcholanthrene on the breast when the method of application did not involve contamination of the skin It was therefore decided to administer the carcinogen by intra peritoneal injection, and 10 mice received 0 25 ml of the almond oil solution by this route The following day all were dead or dying , this was attributed to toxicity of the solvent

A solution of 0 5 per cent methylcholanthrene in arachis oil was therefore substituted, and 0 25 ml was injected intraperitoneally at fortnightly intervals into a group of 12 female IF mice of the 21st and 22nd generations Precautions were taken to avoid contaminating the skin and fur, and if a drop of oil escaped from the needle track on withdrawing the needle, as occasionally happened, it was immediately removed with cotton wool before it could spread The initial age of the mice ranged from 61 to 74 days

Only one of these mice developed mammary carcinoma, a result which nevertheless seems important in view of the fact that none of them survived treatment more than 113 days (mean survival time 102 ± 4 days) In the positive case two mammary tumours were observed on the 105th day in the left abdominal breasts Histologically they belonged to usual types, one being mainly of solid polyhedral celled structure with much squamous metaplasia, the other mainly papillary adenocarcinoma, also with squamous metaplasia It will be observed that the mammary tumours appeared earlier than had been observed in previous experiments, and the result must be regarded as rather fortunate in consequence The early death of the mice in this group seemed to be attributable to direct effects on the peritoneum in nearly all cases there was chronic inflammation with an exudate of "chylous" type, presumably due to the injected oil The most marked reactions were in the region of the hilum of the spleen, where lesions were found which were regarded in three instances as granulomata and in two as sarcomata Pulmonary adenomata were found in only one mouse of this group

Observation of untreated mice

A female IF mouse of the 22nd generation which had received no treatment was found, at the age of 280 days, to have an ulcerated tumour outside the root of the left hind leg On section, the tumour tissue was soft and necrotic Histologically it was a carcinoma, partly adenocarcinomatous and partly solid in structure, and comparable in appearance with mammary carcinoma The site was a possible though unusual one for a breast tumour, and if it was indeed such a tumour it was the first to have occurred in the IF strain As the present experiments were carried out in the belief that mammary carcinoma does not

occur naturally in IF mice, it became important to observe an untreated control group.

All surviving untreated near relatives of the affected mouse were taken. The parents were dead and tumour-free. There were available a sister from the same litter, 2 elder and 2 younger sisters having both parents in common, 7 half sisters (same father), and a few males of similar relationship. There were also set aside some 43 less directly related females. These 55 females and a considerable number of males have been kept under observation but no further mammary tumours have been observed; some of the females are over 500 days old. It therefore seems justifiable to conclude that the tumours induced by methylcholanthrene are not the precocious expression of an inherent tendency.

Summary

Fortnightly cutaneous applications of methylcholanthrene dissolved in almond oil induced mammary carcinoma, frequently multiple, in 30 out of 37 female mice of the breast-cancer-resistant IF strain. Tumours developed in all mice surviving treatment for 126 days or more. The mean induction time was $140 \pm 2 \cdot 9$ days. Pulmonary metastases were found in 2 cases. Pulmonary " adenomata " were found in 19 animals.

Intraperitoneal injections of methylcholanthrene in sesame oil induced mammary carcinoma in one of 12 female IF mice after 105 days. The maximum survival period was 113 days.

A possible breast carcinoma was found in an untreated IF female for the first time. Prolonged observation of a large group of more or less closely related mice has revealed no further example.

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OBITUARY NOTICES OF DECEASED MEMBERS

George Lees Taylor

Born 26th June 1897. Died 9th March 1945

GEORGE LEES TAYLOR, who died on 9th March 1945, at the age of 47, will be remembered as a pathologist who, during the relatively short period of sixteen years, greatly advanced our knowledge in the field of serology, with special reference to the antigens of the red corpuscles in relation to genetics.

He was born at Ashton-under-Lyne and educated at the Manchester Grammar School, where he acquired a knowledge of Latin and Greek, a possession which he cherished throughout his life. As a student of the Faculty of Medicine in the University of Manchester his industry and energy won him distinctions in anatomy, pathology and surgery. After graduation in 1920 he was house surgeon to Sir William Thorburn and house physician to Professor G. R. Murray at the Manchester Royal Infirmary, and obstetric house surgeon at St Mary's Hospital, Manchester. The next seven years were spent in general practice, but in 1929 he decided to return to the laboratory and became a research student in the Department of Pathology in Cambridge. In 1930 he was elected John Lucas Walker Student and in the following five years published, with G. S. and M. E. Adair, a number of papers on the quantitative relations of antigen with antibody in the serum precipitation reaction. His thesis for the Manchester M.D. degree was accepted with commendation and he was later admitted to the Ph.D. degree of the University of Cambridge. In 1939 he was admitted a Member and in 1944 a Fellow of the Royal College of Physicians of London.   In 1935 he was chosen by Professor R. A. Fisher to direct the serological unit of the Galton Laboratory at University College, London.   This appointment gave Taylor an opportunity to apply his great knowledge of serology and particularly of serological methods to the investigation of the antigens of the red blood corpuscles in relation to genetics. Faced with new interests and new problems Taylor applied his originality, ingenuity and scrupulous accuracy to the investigation of genetics by the method of hæmagglutination, and he had already made important contributions to our knowledge of the distribution of the A_1 and A_2 subgroups and of the M and N types when, on the outbreak of war, the Medical Research Council transferred him and his colleagues to the Pathology Laboratory at Cambridge to be responsible for the control and supply of blood-grouping sera for the armed forces and

civilian hospitals. The provision on a large scale of high-titre grouping sera called forth Taylor's gifts for leadership and organisation, and his success in this work of great national importance established the Galton Unit as the laboratory of reference to which problems and difficulties in blood grouping were submitted from all parts of the country. Despite the heavy claims of routine work on his time and energy Taylor continued his researches. Further work was done on the A_1 and A_2 subgroups and on the M and N factors and in 1942 there appeared the first of a series of important papers on the rhesus factor, first reported by Wiener in 1939. With R. R. Race, Taylor described seven allelomorphs of the rhesus factor and four antibodies. The twelve papers published jointly by Taylor and his colleagues on the rhesus factor comprise the most important of his contributions to science and will remain as a memorial of his originality of mind, as of his ingenuity and great technical skill.

The work published in forty papers during the last sixteen years of Taylor's life has advanced our knowledge of serology and especially of hæmagglutination in relation to genetics and his death in middle age and at the height of his powers is a grievous loss both to the science of pathology and to his many friends.

His single-hearted devotion to his work was an example to all of us but, busy as he was, he could always find time to help his friends and the ever-increasing number of colleagues who sought his advice by letter or by visit to his laboratory in Cambridge. He was an excellent teacher and obviously enjoyed his gift for the exposition of difficult problems. This he showed in his written papers, in his spoken communications to societies and on less formal occasions when he was the centre of a group of interested students. His skill as a physician, derived from the years spent in general practice, was always at the service of his colleagues and of members of the laboratory staff, and he was perhaps never happier than when called on to treat their minor ailments. Generous and unselfish in all his undertakings he will long be remembered by everyone whose happiness it has been to count him as a friend.   H. R. DEAN

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Francis George Macnaughton
1891-1943

FRANCIS GEORGE MACNAUGHTON was born in 1891 near Airdrie, Lanarkshire, and was educated at Merchiston College School and Edinburgh University. He graduated M.B., Ch.B. in 1914 and M.D. in 1921, being commended for his thesis. During the war of 1914-1918 he saw service in France and West Africa and on demobilisation worked in the Bacteriology Department of Edinburgh University. In 1921 he went as pathologist to the Clinical Research Institute at St Andrews, founded by Sir James Mackenzie. After two years he went to Leicester and entered general practice. He remained there until his death in 1943. In spite of the cares and duties of a busy practice he still retained a great interest in pathology and its application to medicine. He is survived by a widow, and three children by his first wife.

BOOKS RECEIVED

The bacterial cell in its relation to problems of virulence, immunity and chemotherapy

By RENE J. DUBOS, with an addendum by C. F. ROBINOW. 1945 Cambridge, Mass ; Harvard University Press : London ; Humphrey Milford (Oxford University Press). Pp. xix and 460 ; 21 text figs. and 22 plates. $5.

This important book is the outgrowth of a course of eight lectures delivered in Boston in 1944 and deals with fundamental problems concerning the biochemical architecture of the bacterial cell and its relation to the infectious process. In the opening chapter there is a general discussion of the complexity of the bacterial cell, the biological nature and phylogeny of bacteria and the limitations of the direct and indirect methods used in the study of cellular structure. The chief shortcoming of the indirect approach to cytology, utilising chemical and biological manifestations as guides to the recognition of morphological structures, is that it depends entirely upon the interpretation of results to establish the place of these structures in cellular organisation. But, as the author points out, the history of science provides many examples of the fruitfulness of indirect methods and much of the work discussed in subsequent chapters is concerned with the utilisation of such methods. In the chapter dealing with the cytology of bacteria, attention is focussed on aspects which appear to have a bearing on pathogenicity. Cell granules, nuclear material, spores, cell envelopes and capsules, flagella, cell division and colonial morphology are discussed with emphasis on recent findings obtained by new techniques There is an interesting chapter on the physico-chemical behaviour of bacteria , the reactions of bacteria to stains and especially to the Gram staining technique are discussed at length as cytochemical reactions revealing the probable physico chemical structure of bacteria. But as the author observes later " few cytological reactions possess sufficient specificity to give chemical definition to the objects they reveal ". In subsequent pages there is an account of the cellular structure of bacteria as revealed by enzyme reactions and of the value of serological analysis and bacteriophage action in determining the nature of bacterial antigens and their place in the architecture of the cell. The antigens associated with the flagella and bodies of the salmonella bacilli are considered in detail and some pages are devoted to the immunological and enzymic analysis of the structure of pneumococci, a field in which the author has been especially interested. Variations of the bacterial cell have been considered in relation to the growth cycle and to the effect of the environment on the production of adaptive enzymes and of non transmissible modifications. Discontinuous variation in regard to biochemical activities or antigenic structure would appear, on the evidence available, to arise in most cases by a process of selection of forms normally present in the parent bacterial strain. The need for new experimental methods to obtain accurate evidence on the controversial issues concerning life cycles of bacteria is stressed In a section devoted to the transformation of pneumococcal types it is suggested that this transformation provides an authentic instance of a genetic mutation brought about by a specific treatment and, if the substance which induces transformation is really desoxyribonucleic acid, as the evidence strongly suggests, then nucleic acids of this type must be regarded not merely as structurally important but as functionally active

in determining the biochemical activities and specific characteristics of pneumococcal cells. The nature of virulence has been analysed chiefly in relation to the structure of the bacterial cell, its antigens, toxins and metabolic products, its variability and adaptability, but the importance of the reaction of the host in the infective process is not overlooked. Virulence is not to be considered as a permanent intrinsic property of a given species but "expresses only the ability of a given strain of the infective agent in a certain growth phase to produce a pathological state in a particular host when introduced into that host under well defined conditions". Immunisation against infections is naturally reviewed in relation to the antigens and toxins produced by bacteria, but some space is given to a discussion of the reaction of antibodies with bacteria and their toxins, the preparation of immunising antigens and the appraisal of immunising efficacy. More than sixty pages are devoted to bacteriostatic and bactericidal agents. The mode of action of antiseptics is discussed in relation to the chemical and physical nature of these substances and the chemical groupings and enzyme reactions of bacterial cells. Although a good deal is known about differential susceptibilities of different bacterial groups, as for example the selective action of toxic agents against either Gram-positive or Gram-negative bacteria and the extraordinary resistance of acid-fast bacteria, there are only a few cases in which the chemical composition of the cell can be correlated with susceptibility to a given agent. The author suggests that, although most of the studies of the mechanism of action of antiseptics on bacteria have been concerned exclusively with the inhibition of catabolic reactions, synthetic activities and the process of cell division may in many cases be more susceptible and the first to be affected. Illustrative examples of the action of certain antiseptics on the metabolism of bacteria are given and current views on the mode of action of sulphonamides are discussed. This chapter concludes with sections on the mechanism of drug fastness and on chemotherapeutic agents. In the final chapter on trends and perspectives there is a brief general review of the historical development of knowledge concerning bacteria, and more especially of the nature of specificity and the significance of bacterial variability.

In an addendum on the nuclear apparatus and cell structure of rod-shaped bacteria, C. F. Robinow gives an account of the techniques used and the results of recent cytological studies on bacteria and their spores: there are eight plates of excellent photomicrographs, some of which have already appeared in this author's original papers.

The bibliography, occupying 70 pages, contains over a thousand entries and serves to illustrate the comprehensive treatment of a wide and developing field of knowledge by an author who has himself made considerable contributions to several aspects of the broad subject which he has so ably and critically presented. This is an informative and stimulating book; while it covers a wider field and is rather different in its method of approach, it is a worthy companion to Burnet's "Virus as organism" which appears in the same series of publications.

Introduction to the electron microscope

By F. E. J. OCKENDEN. 1946. London: Williams and Norgate. Pp. 24; 18 figs. on 8 plates and 9 text figs. 2s. 6d.

Electron microscopy is a new and not very easy subject, and there is need for simplified accounts for the benefit of non-physicists. This Monograph of the Quekett Microscopical Club will help to fill such a need, though hardly, we think, in as illuminating a way as might have been adopted. Too much time is spent on more familiar concepts to the dis-

advantage of the less familiar and really important points of comparison with the optical microscope, which are sometimes obscured in half truths and sometimes even expressed incorrectly, as when (p 18) it is said that the equivalent wave length of an electron is proportional to its speed The illustrations, however, are reproduced very well and there is a useful bibliography, but the revolutionary new technique of shadow casting, recently developed by Williams and Wyckoff, apparently came just too late to be included

Pure cultures of algæ   their preparation and maintenance

By E G PRINGSHEIM : 1946   Cambridge   at the University Press. Pp xii and 119 , frontispiece and 8 text figs   7s 6d.

This little book is an exceptionally interesting one of its kind   Whereas the special techniques required for growing bacteria and fungi in pure culture were devised relatively early owing to the practical importance of the organisms to man, the vast assemblage of related forms known collectively as the fresh water algæ have only recently begun to be treated in the same way   It might be supposed that the natural habitats of lake, pond, ditch, stream, puddle, mud or damp rock might be easier to imitate in the laboratory than the natural environment of disease producing organisms   Experience has however belied this expectation and an extreme degree of patience and ingenuity has been required before any but a minute fraction of the organisms involved could be grown in pure culture   That Dr Pringsheim has succeeded beyond the lot of most is partly due to green fingers and a flare for his subject but owes no less to originality and patience   A vast amount still remains to be discovered, but so much helpful practical experience is collected together in this little volume that it should make the culture of algæ a practicable laboratory routine   If it does so, pure science will benefit enormously, for many of these little plants are difficult to study adequately in the field, although of great interest in many connections   It may also be expected that the more orthodox student of the smaller organisms may find a use for some of Dr Pringsheim's shifts and devices in ways not at first thought of   For these alone it is worth possessing   I MANTON

Science versus cancer

By I BERENBLUM   1946   London   Books Ltd   Pp 116 , 14 figs on 8 plates   6s

For the special purpose for which it is written, namely to inform the layman of the known facts of the cancer problem in language which he can understand, this book is well and clearly written, free from exaggeration or sensation   To day there is undoubtedly a popular demand for easily assimilated knowledge in scientific matters of all kinds, medical and otherwise, which, in its sphere, this book should satisfy

The specificity of serological reactions

By KARL LANDSTEINER   Revised edition, 1945   Cambridge, Mass , Harvard University Press   London , Humphrey Milford, Oxford University Press   Pp xiv and 310 , 6 text figs   28s

This revised edition of Landsteiner is almost twice as large as the original   The subject matter has been brought up to date throughout and the whole text has been thoroughly revised , there are few pages of the first edition which have been retained without alteration   In the introductory chapter a few pages devoted to a brief consideration of immunological phenomena and nomenclature are a useful addition   The

chapter on serological specificity of proteins has been considerably extended to include recent work on plant proteins, toxins, bacterial proteins, hormones, enzymes and viruses, and much new material has been added to the chapter on the nature and specificity of antibodies. A new chapter is devoted to detailed consideration of the different in-vitro antigen-antibody reactions and this is followed by the final chapter contributed by Dr Linus Pauling on molecular structure and intermolecular forces. With thorough revision of and addition to the text the bibliography has increased to over two thousand entries; nevertheless, this comprehensive treatise is far from being a dull catalogue of the literature and there can be few relevant papers which have been overlooked in a clear and critical presentation of all aspects of the subject to which the author made such outstanding contributions over many years.

Diagnostic procedures and reagents

Second edition, 1945. New York: American Public Health Association. Pp. vii and 549; 31 text figs. $4.

The second edition of this book purports to "offer to the student a broad concept of the biology of the diseases discussed, to bring to the public health laboratory worker the recent technical practices which are significant in the recognition of those diseases and to provide for the epidemiologist basic information which co-ordinates the laboratory and field investigator". Extensive revision has been carried out and 181 pages of text, comprising nine new chapters, have been added. These chapters, which undoubtedly greatly enhance the value of the book, include chancroid, lymphogranuloma venereum and granuloma inguinale; helminths and protozoa; blood culture; glanders; anthrax; trichinosis; infectious mononucleosis; malaria; and cholera. The book as a whole, however, still lacks balance, as if the authors, in spite of the preface, had not yet decided its scope. On the one hand the reader is guided meticulously through various tests and surrounded by injunctions of the most elementary character, e.g. "Before the plates are inverted, make sure that the medium has solidified" (p. 204); "All glassware, media, etc. are sterile and handled with the usual precautions to prevent contamination" (p. 202). On the other hand, the performance of certain complicated tests, such as the Wassermann is left entirely to individual choice. Again, if the very full consideration given to diphtheria is a measure of what the authors think a laboratory should do, then there are no valid reasons for the omissions in the treatment of other subjects.

The insistence on the need for standardisation in the Wassermann test will meet with general approval, but the discussion of the means to secure this end is inadequate and has little value for the beginner in the absence of precise instructions for carrying out the test. The plea for greater uniformity in the results of various laboratories will not be met until the multiplicity of methods now in use is reduced to a minimum and until laboratories agree to use a standardised procedure in technique and reporting. The American Public Health Association, unencumbered by partisanship and uninfluenced by the dead hand of vested interest, is an ideal body for sponsoring such a procedure.

The value of the book to the epidemiologist is lessened by the omission of all information on streptococcal typing and phage typing of the salmonellæ and staphylococci. All of these procedures have been successfully employed in epidemiological studies and are of the greatest importance; but if they are deemed insufficiently well attested to be regarded as routine measures, they could with advantage be included in an appendix, as is done in the case of "Standard Methods for the Examination of Water

and Sewage " This would at least keep the epidemiologist informed of the
' shape of things to come "

In the treatment of the agglutination reaction in the diagnosis of
enteric fever neither the bearing of the stage of the disease on the results
nor the importance of demonstrating a rising titre, especially in inoculated
subjects is sufficiently stressed The state of the serology of the mannitol
fermenting group of dysentery bacilli is surely not so chaotic as to warrant
complete exclusion from the text The serology of the salmonellae is dealt
with only in a perfunctory manner The chapter on the typing of tubercle
bacilli has been considerably improved by the addition of cultural methods
A statement of how to prepare anthrax infected hides, wool, hair, soil, etc
for examination by cultural and animal inoculation methods would have been
an advantage, as well as of the effect of heat on the spores Glanders, a
comparatively rare disease, has now received notice, but plague and
relapsing fever, which are much commoner, are not mentioned

With the greatly increased scope and usefulness of this edition, the
objectives set out in the preface come much nearer to realisation than
before and there will undoubtedly be a considerable gain in popularity
Books of this nature must of necessity be subject to constant revision
and addition, and doubtless future editions will become more self contained
and show less tendency to refer the reader to outside sources for information
on the latest technical advances and their epidemiological implications

Sternal puncture a method of clinical and cytological investigation

By A PINEY and J L HAMILTON PATERSON Third edition, 1946
London William Heinemann (Medical Books) Ltd Pp xv and 80 , 13
plates (12 in colour) and 2 text figs 15s

A casual glance at this book in a shop would probably lead to a purchase,
for the format is superficially attractive and the coloured plates, when not
closely scrutinised, would suggest an invaluable laboratory companion
But a more detailed inspection would reveal many disappointments and,
to the experienced hæmatologist, many deficiencies The student will look
in vain for a critical discussion on the indications for sternal puncture, an
operation which is not usually performed without definite reason The
house physician, about to carry out his first sternal puncture, will find
himself in trouble if he follows the crude instruction to use a 20 or 25 c c
syringe and expel " the whole of the contents of the syringe " (? 20 or 25 c c
amount not stated) into a watch glass The hæmatologist will find that the
quantitative aspect of marrow cellularity is completely neglected and yet
no qualitative analysis can be complete without reference to the accepted
normal quantitative limits in relation to age Indeed, when viewed from
the utilitarian aspect, the text and plates suggest a greater familiarity with
theory than with practice For example, in three plates illustrating the
marrow in untreated pernicious anæmia, in an early stage of treatment
and at a late stage, the fields are nicely arranged to contain early, inter
mediate and late megaloblasts respectively Yet it is well known that a
megaloblastic marrow may revert to normoblastic erythropoiesis within a
few hours of beginning appropriate treatment The giant *stab* cell is
mentioned, but not illustrated One is informed (p 40) that "hyper
chromia "—a phenomenon which is a mathematical entity depending upon
size and which is never literally visible—is "always seen " These, and
other features, inevitably jar upon those who have practical experience
of sternal puncture examinations Only an uncritical credence could place
confidence in a much padded text which appears to express what ought to
be found in a specimen according to modern hæmatological conceptions,
rather than what is found in practice

Textbook of bacteriology

By Edwin O. Jordan and William Burrows. 14th ed. 1945. London
and Philadelphia : W. B. Saunders Company Ltd. Pp. xvii and 909;
238 text figs. (1 in colour) and 4 plates (2 in colour). 35s.

This well established textbook, appearing after an interval of four
years in its 14th edition, has been brought right up to date. There is
no attempt to confine the book to bacteriology in the stricter sense of the
term. It is rather a treatise which will be valued alike by medical students
and laboratory workers, covering as it does the whole field of invasion of
the human body by parasitic forms. The last 250 pages are devoted to
chapters on medical mycology, medical parasitology, the rickettsias and
the viruses, including bacteriophage. The chapter on medical mycology
is one of the best in the book : that on parasitology includes the metazoal
as well as the protozoal parasites, an unusual feature in a bacteriological
textbook.

The illustrations are numerous and for the most part excellent, including
a number of electron photomicrographs of viruses, bacteriophage, etc.,
and a particularly useful coloured plate illustrating the appearance of
colonies of various dermatophytes on Sabouraud's agar.

The discussion of the applications of bacteriology in industry, which
was a special feature of earlier editions, has been sacrificed to the introduc-
tion of new matter, but the chemical aspects of bacterial metabolism are
extensively discussed in a considerable chapter on bacterial physiology.

Few critical comments need be made. There is no mention of the special
value of sodium desoxycholate in bile solubility tests for pneumococci ;
the photomicrograph of *C. hofmannii* on p. 568 is far from convincing ;
the statement on p. 90 that all attempts to demonstrate H_2O_2 in cultures
of anaerobes have failed suggests that the literature on this subject has
not been very carefully perused ; the statement that *H. pertussis* produces
larger colonies than *H. influenzæ* on Bordet-Gengou medium conflicts with
the experience of many in this country ; there is no mention of the marked
inhibition of *H. pertussis* on heated blood agar, which differentiates so
clearly between recently isolated strains of that bacillus and those of
H. influenzæ.

These, however, are minor criticisms. Altogether this is an excellent
book which will deservedly retain a high place amongst the many good
textbooks of medical bacteriology.

PROCEEDINGS OF THE PATHOLOGICAL SOCIETY OF GREAT BRITAIN AND IRELAND

5th and 6th July 1946

The seventy-second meeting of the Society was held in the University Buildings, Foresterhill, Aberdeen, on Friday and Saturday, 5th and 6th July 1946

Communications and demonstrations

Those marked with an asterisk are abstracted below

R. A. WILLIS. Torulosis.

D. F. CAPPELL and MARJORY N. McFARLANE. Inclusion bodies in the organs of infants.

A. D. MORGAN. A case of amyloidosis of lymph nodes.

A. H. CRUICKSHANK. Observations on bronchiectasis in laboratory rats.

J. C. WHITE. The cytoplasmic basophilia of bone marrow cells.

J. R. M. INNES, A. L. WALPOLE and H. B. PARRY. The effect of alloxan in sheep with and without ligation of the pancreatic duct; comparison of the kidney lesions with the cortical necrosis of lambs due to *Cl. welchii.*

R. J. V. PULVERTAFT and G. LUMB. Antiseptics and bacteriolysis.

G. R. CAMERON, J. H. GADDUM and R. H. D. SHORT. Absorption of toxic agents by the nose

J. CAUICKSHANK. Further studies of bacteria with mobile colonies

R. D. STUART. The lytic property of leptospiral immune serum.

J. A. H. WYLIE. An electron microscope study of leptospiræ.

A. FELIX. Some problems in the Vi phage typing of *S. typhi* and *S. paratyphi B.*

*J. UNGAR. Penicillin esters and their action.

J. F. HEGGIE, T. G. MAGUIRE, M. M. BULL and R. M. HEGGIE. Comparison of the results of the routine standard Wassermann reaction and Kahn test with reference to the introduction of penicillin in the treatment of syphilis.

A. W. DOWNIE and K. R. DUMBELL. The isolation of variola virus on the chorio allantois of the chick embryo.

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J. W. HOWIE. Experimental modification of anthrax spore infection.

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J. S. YOUNG and HARRY D. GRIFFITH. The mechanics of embolic dissemination.

G. A. R. KON, R. J. C. HARRIS and A. HADDOW. Growth inhibitory and carcinogenic effect of derivatives of 4 aminostilbene.

P. R. PEACOCK. Multiple small basal cell carcinomata; their bearing on the ætiology of cancer.

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C. E. Lumsden.   Two rare types of mediastinal cyst : (1) teratomatous cyst of mediastinum ; (2) congenital diverticulum of trachea.

J. S. Young.   Metastatic hypernephroma of thyroid.

W. H. McMenemey.   *(1) Multiple cavernous haemangiomata of spleen.   (2) Hydradenoma of vulva.

Abstracts

615 . 778 (Penicillin esters)

THE ACTION OF PENICILLIN ESTERS

J. Ungar

London

As water-soluble penicillin salts have two undesirable properties—quick absorption and excretion in the urine—it has been suggested that oil-soluble penicillin esters would have certain advantages.   Being water-insoluble, the esters injected in an oil suspension would be slowly absorbed, thus maintaining blood levels for longer periods.

It was assumed that the esters are hydrolysed in the body by an enzyme, esterase, and that free penicillin is continuously liberated.   The early observation of Meyer *et al.* (1943) that the methyl ester of penicillin is hydrolysed in mice was confirmed in my experiments.

Mice injected intramuscularly with an oil suspension of methyl ester showed penicillin levels in the blood and urine.   Mice infected with *Streptococcus haemolyticus* and treated twice daily with penicillin esters survived at the same rate as infected mice treated with sodium penicillin.   There is, however, a species specificity in the ability to hydrolyse penicillin esters.   In addition to mice, guinea-pigs and rats are able to do so, but not rabbits.   Plasma of animals which hydrolyse penicillin esters contains an esterase and this observation makes possible the determination of species which are able to liberate penicillin from the ester.   Human plasma does not contain the esterase and attempts to demonstrate free penicillin in man injected with 75 mg. of the esters (corresponding to 100,000 units of penicillin salt) failed.   Rabbits and human subjects fed with high doses of the methyl ester of penicillin did not show any blood levels of penicillin.   The claim, therefore, that methyl esters or benzyl esters are of value as a method of prolonging the therapeutic effect of penicillin cannot be substantiated.

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PRODUCTION OF ACETYLMETHYLCARBINOL
BY STREPTOCOCCI

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The production of acetylmethylcarbinol by members of the Streptococcus group does not seem to have attracted attention In the present study 20 strains of Enterococcus and 140 strains of Streptococcus were examined for production of acetylmethylcarbinol Only heat sensitive strains (killed by exposure to 60° C for 30 minutes) were included in the Streptococcus group All strains were classified according to their action on 5 per cent oxalated unheated horse blood agar under aerobic conditions Of the enterococci 12 were non hæmolytic (*Streptococcus fæcalis*) and 8 hæmolytic (*Streptococcus durans*), the Streptococcus group comprised 66 beta, 40 alpha and 34 gamma strains

For the Voges Proskauer reaction the organisms were grown in glucose phosphate peptone water (Reps , 1939) and tested on the first, third and fifth day of incubation by the method of Barritt (1936) (The validity of the Barritt test as an indicator of the presence of acetylmethylcarbinol in cultures has been generally accepted and the question as to whether the reaction might be due to metabolites other than acetylmethylcarbinol has not been investigated)

All Enterococcus strains, regardless of their action on blood, gave a positive Voges Proskauer reaction (see Barritt (1936) who found that *Streptococcus fæcalis* gave a positive Voges Proskauer) In the Streptococcus group the reaction was negative in all beta strains and positive in all but one alpha and in all gamma strains The reaction was thus inversely correlated with hæmolysis

The intensity of the V P reaction varied with the strain, strong reactions predominating and being comparable with strong reactions as given by members of the Aerobacter group Weak reactions occurred particularly with strains which grew poorly in the medium The production of acetylmethylcarbinol appears to depend, as in the Aerobacter group (Barritt, 1937) on oxygenation, and to be nil in the absence of oxygen However, exceedingly low tensions of oxygen, compatible with the growth of *B tetani*, may cause a faintly positive reaction Also, when a culture has been grown anaerobically, short subsequent contact with atmospheric oxygen (shaking for one hour) suffices to produce a positive reaction The mechanism of production of acetylmethylcarbinol by streptococci will be discussed in a later publication

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MULTIPLE CAVERNOUS HÆMANGIOMATA OF SPLEEN

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A sixty-five-year-old man, a retired labourer at a machine-tool factory, was admitted to the Royal Infirmary, Worcester, on 17th February 1946 in a state of extreme orthopnœa and died a few hours later. For several years he had had dyspnœa on exertion, with cough, sputum and occasional hæmoptysis. The findings at autopsy included bilateral bronchiectasis with fibrosis of the lungs, terminal pneumonia with abscess formation, anthracosis of hilar nodes, early perilobular cirrhosis of the liver, subacute focal œsophagitis, a solitary hæmangioma of tongue lying posteriorly in the midline, and splenomegaly.

The spleen weighed 909 g. and measured 19·0 × 7·8 × 7·3 cm. It was slate-blue in colour, firm to the touch and heavy, with a slightly thickened capsule. The cut surface revealed several hundred dilated vascular spaces, round or oval, and up to 5 cm. in diameter. Some contained clotted blood or a brown jelly-like material, others liquid blood, venous in colour and more viscid than normal, which dripped on to the slab leaving empty vascular spaces. Microscopically the splenomegaly seemed to be due entirely to the presence of innumerable cavernous spaces and dilated capillaries, mostly filled with erythrocytes or erythrocytes and fibrin, which compressed the normal splenic tissue and tended to obliterate the malpighian bodies. These channels were lined by a flattened endothelium, often ill defined. In several of the spaces innumerable neutrophil leucocytes were noted and attributed to the terminal infection. Infarction was not found and the splenic artery and vein were normal.

The condition is rare, although there are at least 21 recorded instances of similar spleens, most of which are reviewed by Akcakoyunlu (1938) and Pines and Rabinovitch (1942).

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THIS number of the *Journal*, contributed by his pupils, is dedicated to Hubert Maitland Turnbull, D.M., D.Sc., F.R.C.P., F.R.S., on his retirement from the Directorship of the Bernhard Baron Institute at the London Hospital (November, 1906 — October, 1946) and the Professorship of Morbid Anatomy in London University (1919-1946).

It was originally intended that this number should appear at the time of Professor Turnbull's 70th birthday. This proved impossible, but the January number in 1945 celebrated that occasion and contained a copy of his portrait.

HYPERPLASTIC CALLUS SIMULATING SARCOMA IN TWO CASES OF FRAGILITAS OSSIUM

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(PLATES CI-CIX)

THE occurrence of a true neoplasm on a fracture site is an extreme rarity. Lauche (1937), reviewing the literature, remarks that there have been a few authentic cases where tumours (benign or malignant) have arisen on the sites of old healed fractures. This author doubts whether there have been any authentic cases in which the regenerative process of callus production has passed uninterruptedly into a true neoplasm, remarking that in all the recorded cases there is a possibility that the tumour was already present at the time of fracture. In a case where a sarcoma appears to arise immediately on a fracture the possibility that the tumour was present at the time of fracture obviously cannot be excluded. The fact remains however, that, as Lauche remarks, the enormous numbers of fractures produced by war and by road accidents have not given any indication of a tendency to tumour formation at the site of damage. Bearing these facts in mind it is unwise to make the diagnosis of sarcoma arising on a fracture unless the evidence is beyond the possibility of doubt.

The two cases recorded here are of particular interest because both developed enormous masses of callus which closely simulated sarcoma but were proved by the subsequent course of events to be non-malignant. Both were young males suffering from fragilitas ossium, and massive callus simulating bone sarcoma developed in relation to fractures of the femur. The first case (A. E.) has now been followed up for 5 years and the non-malignant nature of the mass has been confirmed. The second case (J. M.) developed a massive callus in

HYPERPLASTIC CALLUS SIMULATING SARCOMA

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

PLATE CII

FIG. 5.—Case 2. X-ray (23.2.44) of left femur 6 weeks after fracture, showing fragile appearance of bone and large mass of callus around fracture site. (Biopsy 2 weeks later.)

FIG. 6.—Case 2. X-ray (29.6.44) of right femur 6 weeks after fracture, showing callus in relation to fracture site.

FIG. 7.—Case 2. X-ray (18.10.44) of right femur 26 weeks after fracture, showing enormous mass of callus filling the thigh and extending up to the neck of the femur. The site of the supracondylar fracture can just be detected. There is also a fracture through (or gap in) the callus opposite the middle of the femur but no signs of disturbance of the continuity of the bone itself.

HYPERPLASTIC CALLUS SIMULATING SARCOMA
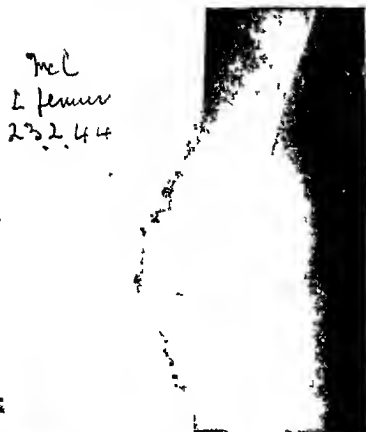
FIG 5

FIG 6

FIG 7.

sarcoma was suspected and a biopsy was performed on 10.3.44, 8 weeks after fracture. An incision was made on the outer and anterior aspect of the thigh about the level of the fracture, and on dividing the muscles a greyish mass was exposed from which a wedge of tissue extending down to the bone was removed ; this is the material here described. Sections were reported as hyperplastic fracture callus and no operative or X-ray treatment was undertaken. The mass continued to enlarge and X-rays showed a steady increase in the callus shadow, and by 20.4.44 a hard swelling could be felt extending from the knee to the inguinal region. A review of the X-rays now showed a further fracture in the trochanteric region and a slipped epiphysis of the femur head which might account for the mass of callus at the upper end of the bone. An X-ray taken on 29.6.44, 24 weeks after fracture, showed callus extending the whole length of the bone. By 1.8.44 (28 weeks) the swelling of the thigh was diminishing ; by 18.10.44 (40 weeks) it had considerably diminished, the œdema and dilated veins had disappeared and an X-ray showed trabeculated porosed callus extending the whole length of the femur. During this period other fractures of the left leg developed—upper end of tibia and fibula (10.3.44), Pott's fracture (3.5.44) ; no excessive callus formed around these. On 20.4.44, while the patient was being moved, a supracondylar fracture of the right femur occurred and an almost identical mass of callus developed in relation to it. Although there was no fracture of the upper end of the right femur the mass of callus extended up its whole length as far as the neck, just as on the left side.

Figs. 6 and 7 show X-rays of the right femur 6 weeks and 26 weeks after fracture. Fig. 7 shows a crack through the centre of the callus but no signs of interruption in the continuity of the bone except the supracondylar fracture. Fig. 8 shows an X-ray of both femurs taken 2 years after the first fracture. The callus is now reduced to a much porosed mass, similar to that in case 1, on both sides.

BIOPSY MATERIAL

Macroscopic. The material from case 1 consisted of several pieces of tissue, some measuring as much as $2 \times 4 \times 1$ cm. ; they had been preserved in formol-saline (4 per cent. formaldehyde) for about 3 months. The bulk of the tissue was almost white in colour, somewhat gelatinous and semi-cartilaginous in consistence ; it resembled tumour tissue but here and there were firmer areas with irregular red-brown markings. The material from case 2 was less abundant but essentially similar, consisting of gelatinous, semi-cartilaginous pale tissue containing firmer reddish areas ; it was fixed in formol-saline (4 per cent. formaldehyde) for about 24 hours. All the tissue could be cut easily with the knife and it was therefore embedded in paraffin without decalcification. Some of the tissue from both cases was kept in the formalin solution and used later for frozen sections. The stains used were Weigert's iron hæmatoxylin and eosin, Mayer's hæmalum and eosin, Weigert's iron hæmatoxylin and van Gieson, Mallory's connective tissue stain, reticulin stain (Gömöri's method, 1937, modified by extending the time of the gold treatment), von Kossa's method for calcium, and Heidenhain's Azan. Frozen sections were stained by Sudan IV and Mayer's hæmalum, methyl violet, and Heidenhain's iron alum hæmatoxylin.

Microscopic

The tissue from both these cases showed essentially the same histological features and a general description will cover both. The pale tissue consisted of œdematous, mucoid and cartilaginous tissue, the appearance of which suggested a chondrosarcoma, particularly

in case 1 where it was more abundant, so that some of the sections contained nothing but this type of tissue. The bulk of this tissue consisted of a fibro-mucoid, cartilage-like substance best classified as " chondroid tissue ". It showed transitional forms grading into true cartilage in some places and in others into woven bone (vide infra).

By selecting suitable pieces of tissue, particularly some from case 2 which passed from the surface to the deeper part of the callus, it was possible to observe the various stages in development of the chondroid tissue and the transitions between it and woven bone. Fig. 9 shows a general view (×8) of such a section from case 2; it consists mainly of chondroid tissue permeated by irregular vascular marrow spaces (pale channels in fig. 9). For convenience of description it can be divided into various zones. Externally (zone A) is the advancing edge of the tissue where it is replacing the muscle and connective tissues of the thigh; zone B shows the early stages of chondroid formation; zone C shows fully developed chondroid with islands of true cartilage and transitions to woven bone; zone D is calcified woven bone. To the naked eye zones A, B and C correspond to the pale mucinous or semi-cartilaginous tissue mentioned above, while zone D is the firmer tissue with reddish markings.

The tissue from case 1 was less regularly arranged than the piece from case 2 here illustrated and it contained larger amounts of the gelatinous early chondroid (zone B), but the various zones could all be recognised in it and material from either case is used to illustrate the appearances described below.

Zone of œdema with destruction of muscle and connective tissue (A). This consists of œdematous tissue with remains of striated muscle in the form of small collections of atrophic fibres separated by œdema. Several small perivascular collections of lymphocytes are seen. Collections of short thick fragments of collagen fibre with square-cut or rounded ends give evidence of destruction of pre-existing connective tissue. Scattered fragments of this, easily visible with Mallory's stain as blue objects darker and thicker than the fibres of the more recently formed collagen, can also be found in zones B and C, some incorporated in the chondroid tissue; scattered atrophic fibres of striated muscle can also be found in zones B and C.

Zone of mucoid œdema, cellular proliferation and early chondroid formation (B). This is œdematous and mucoid tissue containing numerous fusiform and stellate fibroblasts and many plumper cells oval or rounded in form. Fine fibres and fibril brushes are found in relation to these cells, forming a general fine network. Many areas resemble a tissue culture of fibroblasts (fig. 10) and cells in mitosis are not difficult to find. The earliest development of chondroid tissue is seen in this zone (the deeper part of zone B in fig. 9, where strands of darker tissue are seen extending in the direction of the surface). The first sign of this tissue is in relation to groups of cells in which the more rounded types predominate (fig. 11); around these cells

HYPERPLASTIC CALLUS SIMULATING SARCOMA
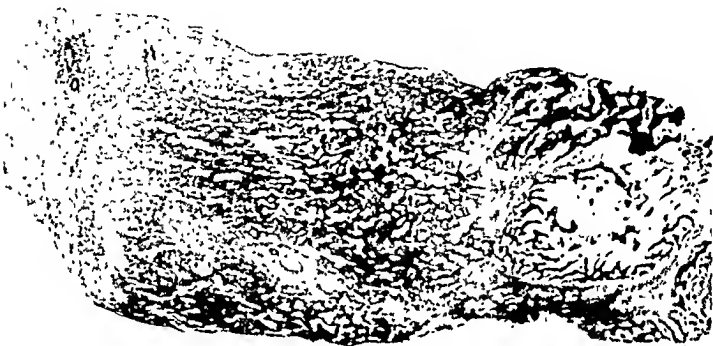
Fig. 8.—Case 2. X-ray (10.1.46) of both femurs about 2 years after the first fracture. The callus has been reconstructed and now forms irregular cancellous masses merging with the much porosed femurs.

Fig. 9.—Case 2. Section of callus showing the various zones described in the text. Weigert's iron hæmatoxylin and van Gieson. ×8.

HYPERPLASTIC CALLUS SIMULATING SARCOMA
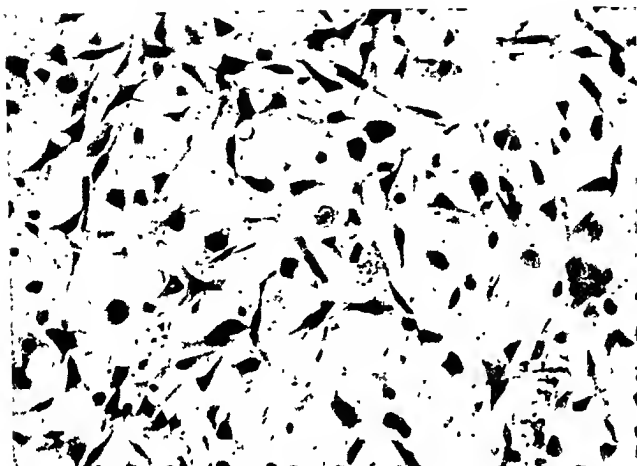
FIG. 10.—Zone B, fig. 9. Frozen section (hæmatoxylin and Sudan IV), showing proliferation of fibroblasts. ×335.
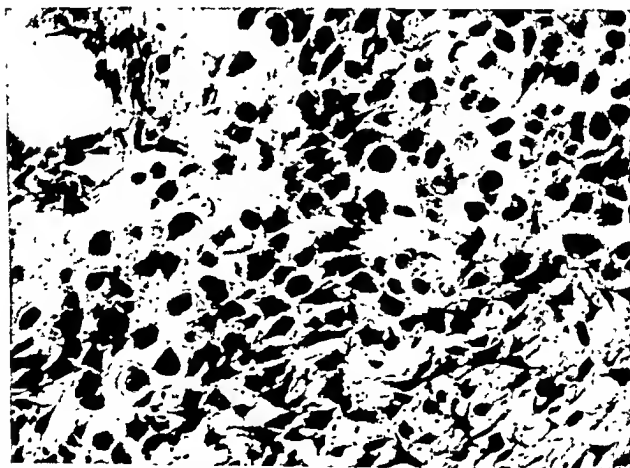
FIG. 11.—The same section as fig. 10, showing a collection of more rounded (chondroblastic) cells. ×335.

there develops faintly basophilic mucoid tissue containing numerous prominent collagen fibres ; many of these fibres tail off into a fine brushwork of fibrils which fan out and become lost in the mucoid material (fig. 13). Another feature of this zone is a marked peri-vascular œdema around newly developed small vessels ; fig. 12 shows a small vessel surrounded by a zone of œdema fluid containing a few scattered cells.

Zone of chondroid tissue (C). This is composed mainly of chondroid tissue permeated by vascular channels which divide it up into irregular masses and strands. It also contains islands and strands of true cartilage. The chondroid tissue as seen here may be described briefly as a fibro-mucoid tissue intermediate in structure between cartilage and woven bone ; all three tissues—cartilage, chondroid and woven bone—occur in this zone, and tissues intermediate in structure between chondroid and woven bone are also seen. The finer structure of these tissues is considered in more detail later. Patchy calcification occurs in this zone, mainly in the centres of the islands of cartilage and in the woven bone ; the chondroid remains for the most part uncalcified or shows only sparsely scattered granules (hæmatoxyphil and von Kossa-positive). The vascular channels form a prominent feature ; they gradually increase in size towards the deeper part, being continuous with the wider marrow spaces in zone D. These channels show one or more thin-walled vessels (arterioles and venules) surrounded by fine reticular tissue composed of fusiform and branching cells with reticulin fibres.

Zone of calcified trabeculæ (D). This is composed of trabeculæ formed mostly of calcified or partially calcified woven bone and to a lesser extent of calcified cartilage, the whole forming an irregular cancellous tissue with vascular (marrow) spaces containing reticular tissue and thin-walled vessels. Scattered osteoclasts are seen on the surfaces of both calcified and non-calcified tissue in zone C and of the calcified trabeculæ in zone D.

MORPHOLOGY OF THE CARTILAGE AND CHONDROID TISSUE

The name " chondroid tissue " is used here for want of a better term. It has been used by histologists for various connective tissues which approach cartilage in structure and usually show vesicular cells in a fibro-mucoid matrix. Part of the tissue might be called " osteoid " were it not for the fact that this term is already used for non-calcified bone tissue which differs from bone only in its lack of calcium. Here the matrix contains less collagen than bone, varying between fibro-mucoid and fibro-cartilaginous and with increasing amounts of collagen passing into a woven bone structure. The cells vary from typical cartilage cells to typical bone cells with intermediate types and in parts of the tissue both cartilage cells and bone cells are found together in a fibro-mucoid matrix. Tissue of this type might

justifiably be called " chondrosteoid ". In describing the morphology of this tissue it will be simplest to describe first the fully developed cartilage seen in the islands and strands noted above, and then the chondroid and stages from this to woven bone.

Cartilage

In the tissue from case 2, of which there was only a small amount, true cartilage occurs as a few scattered islands ; in that from case 1, of which more was available so that larger sections were possible, strands of cartilage form an irregular network connecting up with tracts of calcified trabecular woven bone to form an apparent supporting scaffolding for the general mass, which is composed mainly of chondroid tissue (fig. 14).

The cartilage shows many large vesicular cells lying in a basophil matrix and is very similar to that in the islands of large celled cartilage which are common in fracture callus. Many of the collagen fibres, which pass into the cartilage from the surrounding chondroid, split up into very fine fibres and fibril brushes (as in fig. 13) which fade into the basophilic matrix and in hæmalum and eosin sections this stains a fairly uniform light blue (apart from dark blue areas of calcification) and contrasts with the more eosinophilic, more fibrous chondroid tissue.

Many of the collagen fibres and fibre bundles in the cartilage are in reality masked by the basophil matrix in hæmalum and eosin sections ; they show up partially with iron hæmatoxylin and van Gieson and more completely with Heidenhain's Azan and with Gömöri's reticulin stain. Large fibres and fibre-bundles are often absent in the central parts of the cartilage but with iron hæmatoxylin and van Gieson, Azan and Gömöri's reticulin stain very fine fibres and fibril bundles can be seen, and round many of the cell spaces there is a concentration of collagen fibrils forming a thin collagenous capsule (fig. 15).

Early or more advanced calcification, not sufficient to damage the microtome knife, is usually present in the central parts of the cartilage strands and islands (fig. 17) and appears early in these collagenous capsules.

The cartilage cells vary in size, those with large rounded cell spaces 20-25 μ in diameter being prominent ; there are however many smaller cells, some rounded and others very irregular in shape. Many of the larger cells have basophilic capsules ; these are not usually visible in paraffin sections but show up in frozen (hæmalum-stained) sections as blue lines round the cell spaces. In some of the cells a secondary capsule develops as a lighter blue layer inside the first ; this secondary capsule may increase to fill most of the cell space and form a deep blue sphere or ovoid, the cell then being reduced to only about one-third of the diameter of the original cell space (fig. 20). Frozen sections stained with dilute methyl violet or thionin and mounted in water give good metachromatic staining of the capsules.

HYPERPLASTIC CALLUS SIMULATING SARCOMA
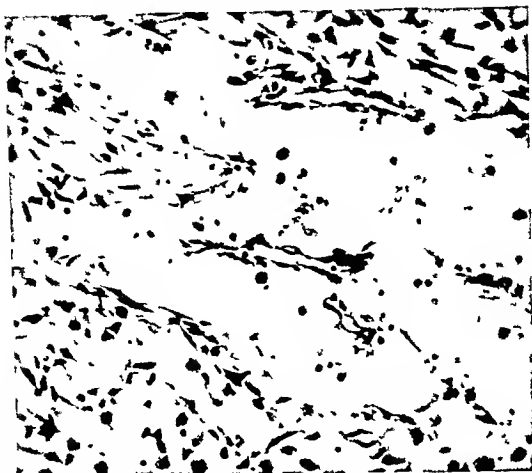
Fig 12 —The same section as fig 10 showing a longitudinal section through a small vessel surrounded by a zone of perivascular œdema   This is the earliest sign of the perivascular (marrow) spaces which are prominent in the more fully developed tissue   ×195
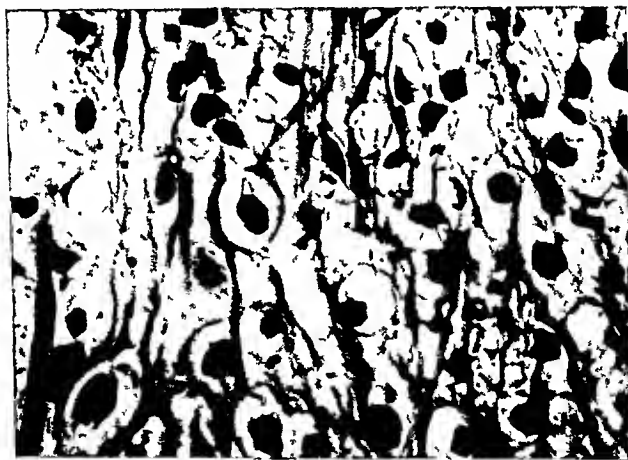
Fig 13 —Callus from case 1   Paraffin section (Mayer's hæmalum and eosin) showing early chondroid tissue with collagen fibres passing in from surrounding œdematous tissue and breaking up into fine brushes and fibril networks which fade into a mucoid matrix   The cell spaces are often outlined by a condensation of these collagen fibres   ×675

HYPERPLASTIC CALLUS SIMULATING SARCOMA

FIG. 14.—Callus from case 1. Paraffin section (Mayer's hæmalum and eosin). The dark-staining network is calcified woven bone (lower part) or cartilage. The lighter tissue is mainly chondroid. ×4

FIG. 15.—Case 2. Collagen capsules of cartilage cells (Heidenhain's Azan). Two cells showing well marked collagen capsules which are connected together by a bundle of collagen fibres merging with them. Fibrils pass on and also connect with the less clearly developed collagen capsule of the cell lower down. ×500.
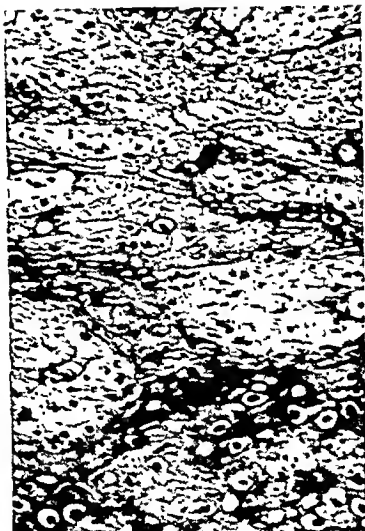
FIG. 16.—Case 1. Chondroid tissue (Heidenhain's Azan), showing strands of fibro-mucoid tissue with perivascular reticular tissue (lighter areas). ×165.

The relation of the collagen capsule to the basophilic capsule is difficult to make out as both cannot be shown in the same section, but they appear to occupy the same position immediately around the cell space and would thus form one capsule consisting of a condensation of collagen fibrils embedded in the basophilic capsular substance. This would fit in with the observations of Roulet (1935) who describes the capsule of the cartilage cell as consisting of a fine net of collagen fibrils ("like a hair-net") embedded in a layer of condensed basophil (mucoid) substance. The small irregular cells show as a rule no signs of a basophilic capsule. In frozen sections (Sudan IV and Mayer's hæmalum) many show fine fat droplets in the cytoplasm as do also some of the large cells.

Chondroid tissue

This differs from cartilage in the absence of marked basophilic staining of the matrix (fig. 18) and the presence of more abundant fibres and fibro bundles. It contains many vesicular cells but very few are capsulated. Their average size is smaller than in the cartilage and many elongated and irregular cells are present.

The chondroid tissue from case 1 contains less collagen fibre than that from case 2, and with iron hæmatoxylin and van Gieson and with Azan shows a network of well stained collagen fibres and fibrils (fig. 16), while round many of the vesicular cells there is a condensation of collagen fibrils outlining the cell space.

In the chondroid tissue from case 2 the collagen fibrils and fibre bundles are closer together and in places merge to form a fairly uniform red background with van Gieson. Where there is most collagen most of the cells are stellate in shape and numerous calcium granules are present, so that the structure is that of partially calcified woven bone (fig. 17).

The cell types found in the chondroid and in this "chondrosteoid" tissue are of interest. In paraffin sections (hæmalum and eosin and iron hæmatoxylin and van Gieson) one can distinguish at once two main types of cell, the rounded and the stellate; in addition there are many elongated, dumb-bell and twisted bizarre shapes. The morphology of these different cell types can best be studied in frozen sections where shrinkage is minimal. These were stained with Sudan IV and Mayer's hæmalum and also iron alum hæmatoxylin; it was found that if the alum hæmatoxylin was only slightly differentiated in the alum solution diluted with equal parts of water, the whole of the cytoplasm including the cytoplasmic processes of the stellate cells could be demonstrated.

The rounded cartilage-type cells may fill the cell space or they may appear to fill only the central part, being, however, usually connected to the wall of the space by fine threads. A non-staining fluid collects in this outer part of the space and this fluid may acquire basophilic

PLATE CVII

FIG. 17.—Case 2. Cartilage, chondrosteoid tissue and woven bone (Weigert's iron hæmatoxylin and van Gieson). Above is large-celled cartilage with early calcification (dark granular masses around cell spaces). This passes below into a tissue which resembles woven bone except that it is more mucoid and less collagenous. The dark zone round the lateral and lower margins of the central perivascular space is more collagenous and is calcified, thus showing the structure of calcified woven bone. ×120.

FIG. 18.—Case 1. Cartilage and chondroid tissue (Mayer's hæmalum and eosin). In the centre is a vascular space with vessels and perivascular reticular tissue; to the left is a meshwork of lightly staining chondroid tissue. The deeply staining tissue is partly calcified large-celled cartilage with strongly basophilic matrix. ×40.

FIG. 19.—Case 1. Cartilage cells showing external and early internal capsules (Sudan IV and Mayer's hæmalum). The cell in the centre shows a thin external capsule and a partly developed internal capsule, slightly basophilic except in its lower left quadrant. Another cell (bottom left) shows a basophilic external capsule and a pericellular space containing clear (not yet basophilic) substance. ×750.

FIG. 20.—Case 1. Cartilage cell with external and internal capsule (Sudan IV and Mayer's hæmalum). There is a wide strongly basophilic external capsule and a well developed basophilic internal capsule fills the pericellular space; this showed very fine radial striations which are not visible in the photograph. ×750.

FIG. 21.—Case 2. Osteocytes showing cell processes (Heidenhain's iron alum hæmatoxylin). The cytoplasm is stained an intense black; fine cytoplasmic processes pass out in all directions but cannot be followed far in the photograph, as they pass out of focus. The clouds of granules are early calcification. ×990.

PLATE CVII

HYPERPLASTIC CALLUS SIMULATING SARCOMA

Fig. 17.

Fig. 18.

Fig. 19

Fig. 20.

Fig. 21

HYPERPLASTIC CALLUS SIMULATING SARCOMA

Fig 22—Case 2 Cartilago cells and cell with arborescent processes Heidenhain's iron alum hæmatoxylin. ×750

Fig. 23.—Case 2. Osteocyte with cytoplasmic processes close to a cartilage cell. The granular background is early calcification in the matrix. Heidenhain's iron alum hæmatoxylin. ×1100.

Fig 24 "" trabecular of fracture of (dark) are seen in the cancellous spaces. From a 61-year-old female. ×60.

changes are occurring in the muscle and connective tissue leading to their dissolution, and the oldest is seen in the deepest part, where a calcified, cancellous structure has replaced the normal tissues. The appearances suggest that the hyperplastic callus in these two cases passed through the following stages. (1) An œdema of the muscle and connective tissue similar to an inflammatory œdema and presumably determined by changes in the vascular conditions of the region; (2) atrophy of the muscle and connective tissue, in place of which develops (3) an œdematous mucoid tissue containing numerous cells of fibroblast type. These cells differentiate in various directions; many of them produce (either directly or by their action on the surrounding protein substrate) bundles, brushes and webs of collagen fibrils; some assume a rounded shape (chondroblasts) and become surrounded by increasing amounts of mucoid tissue, thus forming a fibro-mucoid (chondroid) tissue or, with increased development of basophil mucoid masking the fibres, true cartilage. Other cells remain stellate in shape with long cytoplasmic processes and become included in the fibro-mucoid; these develop into the bone-type cells in chondrosteoid tissue which, with increasing amounts of collagen, assumes the characters of woven bone. In the zones of œdema immediately around vessels neither collagen nor mucoid develops, but a fine reticulin network associated with scattered stellate cells forms the earliest stage of the perivascular (marrow) spaces.

The development of the cancellous tissue (zone D, fig. 9) is brought about by osteoclastic erosion on the edge of the marrow spaces, which are enlarged at the expense of the chondroid, chondrosteoid and cartilage. Meanwhile the more collagenous parts of the chondrosteoid tissue and the central parts of the strands of true cartilage have calcified, so that after remodelling by osteoclasts, trabeculæ composed of calcified woven bone, and also to a lesser extent of calcified cartilage, are produced. On these, new bone is now laid down by the osteoblastic cells on their surfaces.

There appears to be little doubt that the above is the sequence of events. The one point which is doubtful is the source of the young fibroblastic cells which occupy the mucoid and œdematous tissue. Do they develop from the local soft tissues or have they spread out from the bone or periosteum in the form of a growing edge to an advancing mass of callus? This question is discussed below.

DISCUSSION

Many interesting points arise from consideration of these two cases. I have been unable to find any detailed account of a closely similar case in the literature. Brailsford (1943) however gives an account of the radiographic appearances of four cases of osteogenesis imperfecta in which masses of bone were found in relation to the femur. He gives an illustration of the X-ray appearances of one of

these showing involvement of the lower two-thirds of both femurs in masses of spongy bone very similar to the end result seen in case 1 here reported. He assumes that this condition resulted from subperiosteal hæmorrhages produced by scurvy, but the evidence for this conclusion appears very scanty and the right femur shows a bend suggesting an old fracture in the lower third of the shaft and also an irregular mass of bone extending into the soft tissues from the upper half of the shaft. One of the other three cases he mentions was a boy of 14 who showed a cancellous mass extending from the lesser trochanter down the whole length of the diaphysis of the right femur. This developed after a contusion of the thigh 7 years previously. It appears to me that all four of Brailsford's cases may well have been traumatic in origin, with, quite possibly, incomplete fractures which passed unnoticed at the time.

In one of these four cases Brailsford notes that there was also a mass on the lower end of the right tibia and that this underwent a rapid increase in size and showed evidence of " malignant metaplasia ". The subsequent history of this case is not recorded so that it is not clear that this was in fact malignant; it may have been a rapid callus production following trauma to the mass. I can only find one case recorded where a proved sarcoma occurred in fragilitas ossium. This is a report by Jewell and Lofstrom (1940) of a man of 49 with familial bone fragility who developed a sarcoma of the pelvis; but there was no evidence that this was directly connected with a fracture.

Had the leg been removed in my case 1 it would have been impossible to prove that it was not a sarcoma cured by amputation. It was fortunate that sections extending from the surface of the callus to the deeper part of the tissue could be examined; had the biopsy material been limited to the surface layers an accurate histological diagnosis would probably have been impossible. This applies particularly to the material from case 1, where "chondrosarcoma" was, I think, a not unjustifiable diagnosis from the first sections which I examined. This diagnosis was corrected only by a careful consideration of the clinical and radiological features and by examination of more of the biopsy material which included some of the deeper parts of the mass.

Case 1 presented the greater difficulty because of the large amount of mucoid œdema and chondroid tissue, which was quite unlike the reaction in the specimens of normal fracture callus which I had seen. Fig. 24, for instance, shows the edge of a callus round a 2-week-old fracture just below the middle of the femur. Here there is no appreciable œdema, woven bone is developing on the surface in a fairly dense connective tissue matrix, while, below this, vascular marrow spaces filled with fine reticular tissue and remains of striated muscle separate the trabeculæ of woven bone.

The histological points which weighed most with me against the

diagnosis of sarcoma in the material from case 1 were (1) the presence of trabeculæ of well formed calcified woven bone in the deeper part of the tissue, with gradations from this to the more superficial chondroid tissue and thence to the very cellular fibro- and chondro-blastic tissue at the surface, and (2) the system of spaces running roughly at right angles to the surface and containing the blood vessels lying in a perivascular reticular tissue—an arrangement I have never seen in a sarcoma.

After the experience of case 1 the material from case 2 presented less difficulty ; there was less of the very mucinous type of chondroid tissue and the well developed woven bone (zone D, fig. 9) and system of vessels with perivascular reticular tissue justified an immediate diagnosis of " hyperplastic callus ". Viewing the cases in retrospect one can perhaps say that a neoplasm showing such rapid and massive involvement of muscle would show clearer cytological evidence of malignancy. This however was not a point to which much weight could be attached when the first case was seen.

Since hyperplastic callus as massive as that here described must be very rare and since fragilitas ossium is not common, and since massive callus developed in both thighs in case 2, one is inclined to think that there must be some connection between this bone defect and massive callus formation, but apart from the paper by Brailsford quoted above I have found nothing in the literature bearing on this point. It is generally agreed that osteogenesis imperfecta and fragilitas ossium are different grades of the same defect, namely a congenital hypoplasia of bone tissue. In spite of this defect fractures, which occur in large numbers with little or no trauma, heal easily (Sonnenschein, 1938), so that the stimulus to bone production by a fracture overcomes, at least to a sufficient extent for healing, the osteoblastic inertia. Dietrich (1929) discusses the pathology of osteogenesis imperfecta and fragilitas ossium with copious references to the literature up to 1928 ; he discusses and illustrates the healing of fractures in this disease but makes no mention of massive callus production. Lauche, in discussing the pathology of " callus luxurians ", makes no mention of its occurrence in fragilitas ossium. He does however make some remarks as to the conditions which are believed to predispose to its development, namely (1) much shattering of bone, *e.g.* gun-shot fractures with tearing and crushing of soft parts ; (2) infection, especially with compound fractures ; (3) its more frequent occurrence in the upper third of the femur and humerus shaft where failure of fixation with hæmorrhage and tearing of tissues may be important factors. (4) Bier (quoted by Lauche) thought that escape of bone marrow or joint fluid into the tissues was responsible for the production of massive callus, but Lauche found no evidence for this and the experimental injection of joint fluid into tissues does not stimulate bone formation. (5) Frangheim and Orth (both quoted by Lauche) have seen cases where no cause could be

found and have postulated an "individual predisposition". Considering the bearing of the above on these two cases, there may have been sufficient violence to produce muscle damage and hæmorrhage with the spiral fracture of the femur shaft in case 1; in case 2 however there was no violence with either of the fractures and damage to soft parts must have been minimal. Each case had had dozens of fractures previously and, as noted above, case 2 sustained fractures of the tibia and fibula during the period of development of the hyperplastic callus in the thighs; in none of these was there any evidence of excessive callus formation. There was however no evidence that either case had had a previous fracture of the femur and, taking into account Brailsford's cases, there does appear to be a connection between osteogenesis imperfecta and excessive bone formation in relation to fractures or other lesions—probably traumatic —of the femur.

The literature contains little information concerning the histology of hyperplastic callus; Lauche states that microscopic examination shows nothing noteworthy except the frequent formation of numerous islands of cartilage.

The present cases raise the question whether the great production of chondroid tissue is related to the very rapid growth of the callus or whether it may be largely dependent on the defective osteogenesis, which might encourage the production of an excess of cartilage in place of bone. I have recently obtained material from a case of osteogenesis imperfecta in a female infant aged 17 days (case 3) which showed innumerable healing fractures; most of these showed cartilaginous as well as bony callus. Is this cartilage more abundant than in fractures of normal bones? If so is the excessive cartilage directly related to the bone defect or is it only the result of the undue mobility of these unsplinted fractures? As regards the amount of cartilage to be expected in the fracture callus of normal human bones, there is no doubt that islands of cartilage are frequently found, and in healing fractures at certain sites, e.g. ribs, cartilage is nearly always present. Lauche in discussing cartilaginous callus makes the following points. (1) It was formerly believed (Ollier et al., quoted by Lauche) that all fractures passed through a stage of cartilaginous callus but a more careful study, particularly of material from fractures where healing had been undisturbed, showed that under ideal healing conditions no cartilage appears. (2) Animal experiments by many workers (lit. given by Lauche) have shown that cartilage forms when there is sufficient shearing movement between the fractured ends of the bone during callus formation. (3) That this view applies to human fractures is shown best in rib fractures, which almost always develop cartilaginous callus during healing and where the unidirectional respiratory movement results in the development of regularly arranged cartilage, surrounding the fracture site as a ring or collar. If the line of fracture is oblique or irregular, so is the cartilage deposit, and the

HYPERPLASTIC CALLUS SIMULATING SARCOMA
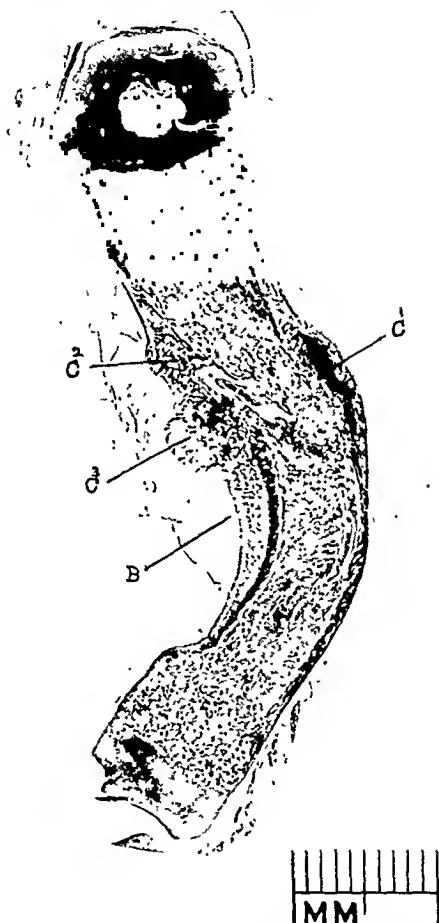
FIG. 25.—Tibia from a case of osteogenesis imperfecta; female aged 17 days. There
has been a fracture about the middle of the shaft and a collar of cartilage, seen at
C¹, C² and C³, has developed in the callus. There has also been a bending of the
lower half of the shaft, the concavity of which is buttressed by new periosteal
woven bone (B). Mayer's hæmalum and eosin. × 2·5.

dependence of the formation of cartilage upon the stimulus of movement between the bone ends can be clearly recognised. Lauche (pp. 242-244) gives some good illustrations of sections of rib fractures with different grades of displacement and at different ages and all show this characteristic arrangement of the cartilage deposits in the callus. Now the cartilage deposits in the fractures of my case of osteogenesis imperfecta show exactly the same sort of arrangement as in Lauche's illustrations. Fig. 25, a longitudinal section of the fractured and bent tibia from my case, shows masses of cartilage (C^1, C^2, C^3) on either side of the fracture site, corresponding to the opposite halves of a cartilaginous collar surrounding the broken end. On the right the collar is a continuous sheet of cartilage (C^1), on the left the cartilage (C^2, C^3) is separated somewhat by the development of a small intervening strip of trabecular woven bone. This section also shows a marked " buttressing " of the concavity of the bowed bone by a periosteal deposit of trabecular woven bone (B). The whole picture corresponds closely to Lauche's fig. 30, showing a bent fractured rib in a child of 4 months with a similar cartilage collar and buttressing of the concavity by new spongy bone. On such evidence as this therefore we can, I think, say that, apart from the question of the total amount of bone produced, the bones in osteogenesis imperfecta react normally to the stimulus of a fracture and that the amount of cartilage in the fracture callus is no greater than could be expected in a non-immobilised fracture in a normal bone.

Apart from the quantity of cartilaginous callus produced in case 3 there is a qualitative difference in the tissue as compared with that in cases 1 and 2. In case 3 there is fully developed vesicular-cell cartilage in well defined masses showing a fairly sharp transition to surrounding woven bone ; there is no chondroid or chondrosteoid tissue such as is found in cases 1 and 2. It seems clear therefore that the peculiar features of this hyperplastic callus cannot be attributed to the essential bone defect in these cases and must be dependent on the very rapid growth of the callus.

It is of interest to consider how such a mass of new tissue develops and rapidly replaces the muscle and connective tissues of the limb. There appear to be two possible modes of development, namely (a) an outgrowth of a mass of cells, derived from the periosteum and fractured bone ends, which extends like a tumour and infiltrates and replaces the normal tissues or (b) the spread of a reaction (presumably initiated by a chemical stimulus of some sort) which extends like an inflammatory process. The histological appearances of the advancing edge of this hyperplastic callus suggest the spread of a reaction involving vascular dilatation and œdema followed by atrophy of the muscle and collagen fibres, then a rapid proliferation of fibroblasts, some of which by slight functional and morphological modifications differentiate into chondro- and osteoblasts. Under the influence of these cells mucoid and collagenous matrix is formed. The

appearances are those of tissue dissolution followed by the evolution of new tissues.

It would be possible to interpret this picture as a rapid spread of vascular fibroblastic tissue from the bone into the soft tissues with subsequent differentiation of the fibroblasts, but there are difficulties in this view. Fibroblasts do not spread out into tissue in this way unless there is a substrate of blood clot or damaged tissue to be organised. We can explain the healing of a fracture by the organisation of the local blood-clot and damaged tissue with subsequent differentiation of some of the fibroblasts to form bone and cartilage, but it would be difficult to explain the extensive spread of callus seen in these cases on such a basis. If there had been extensive hæmorrhage throughout the thigh muscles in these cases, organisation of those parts of the clot more distant from the bone would surely have taken place locally, with the production of ordinary scar tissue before an advancing edge of fibroblasts emanating from the bone could have reached the spot. In fact there was no evidence of such extensive hæmorrhage, either as discoloration of the limb or histologically.

Another strong piece of evidence for the existence of a process of callus formation which is not dependent on the wandering out or growing out of cells from the bone or periosteum is the occurrence of myositis ossificans following injury or inflammatory damage to muscle without bone injury. Dittrich (1926) remarks that the production of bone under these circumstances may be regarded as a regeneration of connective tissue with altered differentiation resulting from altered local conditions. Callus which extends into soft tissues and includes within it remains of striated muscle is often called " parosteal callus " (Lauche), but, judging from my own (admittedly not very numerous) samples from healing fractures, I should say that virtually all external callus extends into the muscle and connective tissue attachments to the bone and shows remains of muscle fibres incorporated within it. Some extension into parosteal tissues is therefore a normal event in callus formation; it involves however only the local tissues, which may well have been the site of hæmorrhage or traumatic œdema or both, and it could be considered as part of a normal process of organisation by cells of endosteal or periosteal origin. Such an explanation will not however cover myositis ossificans without bone damage, nor is it adequate for this hyperplastic callus developing in parts at some distance from the bone.

Evidence for the existence of a chemical " organiser " which may eventually furnish an explanation of such heterotopic cartilage and bone formation has recently been published by Levander (1938) and Levander and Willstaedt (1946). These workers have described the experimental production of cartilage and bone by the injection of alcoholic extracts of bone into the muscles and they are now attempt-

ing to isolate the substances responsible for this reaction. Lacroix (1945) reports similar results from the injection of alcoholic extracts of cartilage.

SUMMARY

1. Two cases are described showing massive hyperplastic fracture callus which closely simulated sarcoma but was proved by the subsequent course of events to be non-neoplastic.

2. Both patients were boys with fragilitas ossium and innumerable previous fractures which had healed without excessive callus.

3. The hyperplastic callus developed in femoral fractures and in the second case a subsequent fracture of the opposite femur produced a similar state of affairs.

4. A description is given of the histology of the biopsy material, a striking feature of which was the formation of much fibro-mucoid tissue (" chondroid ") intermediate in structure between cartilage and woven bone.

5. The formation of callus in tissues away from bone is discussed and it is concluded that it cannot be satisfactorily explained by outgrowth of cells from the bone or periosteum.

6. It is suggested that the production of a chemical " organiser " such as is foreshadowed in recently published work by Levander and Willstaedt may be found to furnish an explanation.

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CALCIFICATION OF THE LUNG ALVEOLI

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(PLATES CX-CXVI)

THE deposition of calcium in normal tissues is unusual. It is commonly found, however, in the neighbourhood of degenerate, dead and chronically inflamed tissues. It may be found in diseases involving a general upset of calcium metabolism such as rickets, but, even in these, extensive calcification of otherwise normal lungs is extremely rare. In the present communication two examples of widespread calcification of otherwise normal lungs are described and reference is made to similar calcification associated with organised bronchopneumonia and to calcification of the lungs in a hen. The other feature presented in each of these instances was disease of the kidneys

Case 1

Summary of history. The patient was a lank thin man aged 62 years. He was admitted to St Thomas's Hospital in December 1942 complaining of constipation, pains in the stomach and, for 4 or 5 weeks, pains in the legs while walking. Swelling of the ankles and palpitation had been present for a few weeks. He had also noticed swollen glands in his neck and he was treated by his doctor for tonsillitis, after which he developed stomatitis. There was increased frequency of micturition, some loss of weight and generalised pruritus. On examination he was found to be a very deaf, mentally dull patient. Slight jaundice was present. The heart was not enlarged; a systolic murmur was heard at the apex. The pulse was 60 per minute, blood pressure 110/55 and temperature 98·6° F. The respiration rate was 20 per minute and the lungs were emphysematous. The tongue was furred and the mucous membrane of the mouth covered by flakes of yellowish exudate, while the tonsils were enlarged and the fauces inflamed. Teeth were missing. Examination of the abdomen revealed tenderness in the upper part, especially under the costal margins. The liver was enlarged and tender. The spleen and kidneys were not palpable The urinary bladder was distended. A small hard irregular lump was felt in the posterior part of the prostate. The pulse and respiration rate rose rapidly and the patient died on the fifth day after admission.

Summary of post-mortem findings. Heart: 13½ oz., patchy fibrosis of left wall extending to interventricular septum. Severe atheroma of coronary arteries and less severe general atheroma. *Lungs:* right 36 oz , left 28 oz., severe œdema of both lungs, which were rusty-

brown, red and tough, with a firm cut surface. This showed a reticular pattern of fine-meshed delicate white lines distributed in patches, most marked beneath the pleura but also in the depths of the lungs. *Peritoneum*: numerous delicate adhesions over matted coils of small intestine and over liver and spleen. *Liver*: 63 oz., indefinite greyish-white infiltration of portal systems in jaundiced liver. *Spleen*: 6 oz., soft and purplish grey. *Kidneys*: right 6½ oz., left 6¼ oz., the left kidney showing a swollen pale cortex with cloudy pattern and sharp demarcation between cortex and medulla; less marked change in congested right kidney. *Tonsils*: smooth greyish-white infiltration of both. *Lymphatic glands*: greyish-white infiltration of angular cervical and coeliac lymph glands, also of those in base of mesentery and around pancreas. *Stomach*: two small nodules in submucosa. *Prostate*: round, firm white carcinomatous nodule 1·5 cm. in diameter in otherwise normal prostate. *Bones*: no naked-eye changes noted. *Brain*: not examined.

Histological examination. *Heart*: patchy fibrosis of myocardium and calcification of myocardial fibres and intima of blood vessels (figs. 1 and 2). *Lungs*: severe calcification of alveolar walls and of walls of many small arteries. In many places the appearance is as if the capillaries of the alveolar walls are embedded in a mass of calcified material. Von Kossa's silver method gave a positive reaction (figs. 3-5). Elastic fibres appear to be decreased in number and those present are unusually straight and often fragmented. *Kidneys*: slight chronic nephritis; calcification of many tubules and a few glomeruli. *Tonsils*: reticulum-cell sarcoma (figs. 6 and 7). *Lymphatic glands*: reticulum-cell sarcoma. *Stomach*: reticulum-cell sarcoma in submucosa and mucosa. *Spleen*: sarcomatous infiltration and fibrosis around medium-sized arteries. *Prostate*: tubular columnar- and cuboidal-celled carcinoma (fig. 8). *Liver*: acute hepatitis. All the portal tracts are infiltrated by numerous polymorphonuclear leucocytes and mononuclear cells; the cellular infiltration is especially marked around the bile ducts, some of which contain inflammatory cells in their lumen. There is also inflammatory-cell infiltration among the liver cells, especially in scattered foci. The finest bile capillaries are distended with bile. Many of the liver cells contain two nuclei; others have large dark-staining nuclei and their cytoplasm often takes up eosin more intensely than the cells with normal nuclei.

The calcified parts of the lungs had a brick-red appearance naked-eye, with a fine honeycombed cut surface suggestive of porous brick. On passing the thumb lightly over this cut surface an unusual resistance and slightly gritty feeling were apparent. Although tough and resembling a " heart-failure " lung, the tissue could be readily cut with an ordinary knife and calcification was not diagnosed until sections had shown its presence. The calcification in the myocardium was not confined to the infarcted and fibrotic areas, though in the main it may have been secondary to these lesions. The " chronic "

PLATE CX

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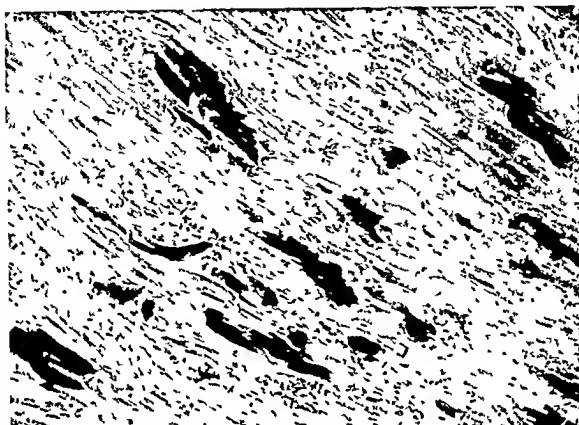
Fig 1—Case 1   Section of myocardium of left ventricle in the region of a patch of
fibrosis, showing calcification of muscle bundles   Von Kossa's method   ×95
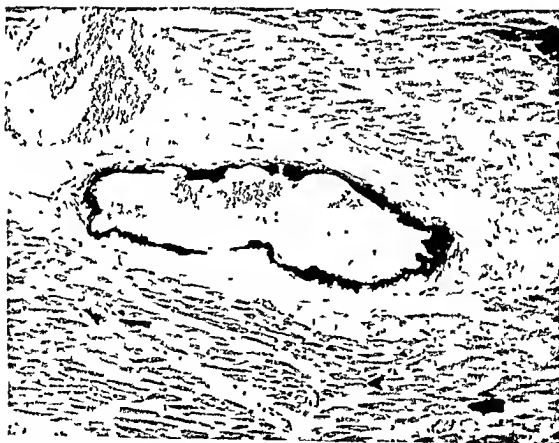
Fig 2—Case 1   Calcification in myocardium, showing infiltration of the greater part
of the wall of a blood vessel   Von Kossa's method   ×95

PLATE CXI

CALCIFICATION OF THE LUNG ALVEOLI
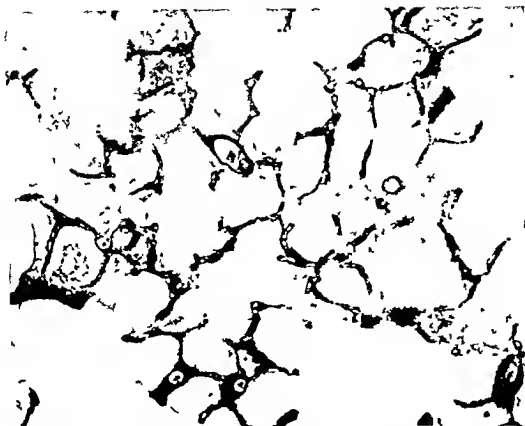
FIG 3 —Case 1   Low power view of lung, showing calcification of the alveolar walls
and absence of inflammatory reaction   Von Kossa's method   × 35
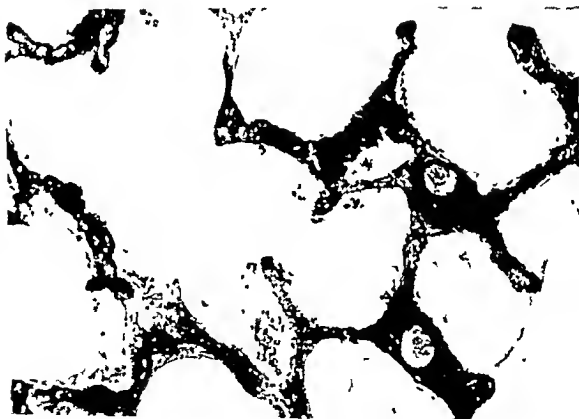
FIG 4 —Case 1   Higher power view, showing calcification in alveolar walls   Von
Kossa's method   × 95

nephritis was little more than that found as an age change, and calcification was most marked in the atrophied fibrotic parts. In the lungs, however, there were no lesions suggesting a previous inflammatory, degenerative or fibrotic change.

The carcinoma of prostate was small and localised and there were no apparent bony or other secondaries. Neither were there obvious bony secondaries from the reticulosarcoma. No special search, however, was made at the post-mortem examination for bony secondaries.

The parathyroids were not investigated, but it is improbable that they were materially enlarged; otherwise they would have been noted on examining the glands and neck.

Case 2

Summary of history. The patient was a woman aged 48 who complained of an abdominal mass and severe uterine hæmorrhage. At operation " a large, necrotic, hæmorrhagic and cystic tumour " was removed from the pelvis. This was reported as a fibromyoma. Six months later the patient returned to hospital with a recurrent tumour reaching from the pelvis to the umbilicus and causing intestinal obstruction. A colostomy was performed and the recurrent growth was found to be a spindle-celled myosarcoma. The patient died two months later.

Summary of post-mortem findings. A thin wasted woman, with distended abdomen and a colostomy opening in the left flank. *Abdomen :* matting of coils of small intestine over a largely necrotic cystic mass extending from pelvis to umbilicus. No infiltration of intestinal or uterine walls. *Ovaries :* not identified. Small secondaries of firm greyish-white tissue, having a smooth cut surface with a whorled pattern resembling fibromyoma, were scattered over the peritoneum, especially of the anterior abdominal wall above the urinary bladder; confluent plaques were present on the abdominal surface of the diaphragm. Similar secondaries were present on the pleural surface of the right side of the diaphragm and on the visceral and parietal pleura over the lower lobe of the right lung. *Heart :* simple atrophy of myocardium. *Arteries :* very slight general atheroma. *Lungs :* patch of rough honeycomb calcification in lower lobe of right lung and in apex of lower lobe of left lung. Secondaries in the pleura were directly infiltrating the lower lobe of the right lung. No bronchopneumonia. *Liver :* fatty change. *Spleen :* small and soft. *Kidneys :* narrowed cortex and slight dilatation of pelves. *Urinary bladder :* acute cystitis of plum-coloured, œdematous, thick-walled urinary bladder, with coating of grey calcareous material on mucosa. *Veins :* thrombosis of left femoral vein.

Histological examination. This revealed leiomyosarcomatous growth, chronic nephritis and calcification of the lungs as the chief findings.

In the lungs, the affected alveolar walls were thickened and

infiltrated by calcified material in granular form and also in the form of rod-like masses in some of the alveoli. This material appeared not only in the alveolar walls but also within the alveoli near the walls. There was no evidence of old or recent inflammation in these areas (fig. 9).

Calcification associated with organised bronchopneumonia

The association of calcification with the inflammatory reaction is more commonly found and, for comparison with the cases above-described, a note of a case of this kind is included. A boy of eight years died of streptococcal tonsillitis, bronchopneumonia and subacute nephritis. The lungs showed severe calcification of the alveolar walls, with partially organised pneumonia filling the alveoli (fig. 10) and a severe subacute nephritis or late intermediate stage of nephritis acris.

This phenomenon is not confined to human beings and a somewhat similar condition was found in a fowl about ten months old. It was a black leghorn and was with some ten sisters in an open run. For about a month it was very much less active than the others; it ate from the feeding trough but stood about moping most of the day, although if approached it would move away briskly. As it did not improve, was losing weight and had given up laying, it was killed.

The kidneys showed a severe subacute to chronic nephritis (fig. 11) (late intermediate stage of nephritis acris), while the lungs were the seat of calcification in all respects similar to that seen in cases 1 and 2 (figs. 12 and 13). There was also slight calcification of the myocardium (fig. 14).

DISCUSSION

As the parathyroids were not examined histologically in any of these cases and as neither the calcium nor the phosphate content of the serum was investigated it cannot be considered that any of them has been fully investigated. Widespread calcification and particularly calcification of the lung alveoli is, however, of such general interest that attention should be drawn to its occurrence. The boy with bronchopneumonia and the fowl both had a definite active nephritis; in the other two cases the nephritis was slight. Hild (1942) described, from the radiological appearances, changes in the lungs suggesting alveolar calcification. This was in an infant who had renal failure and a raised level of serum phosphate. Smyth and Goldman (1934) described "deposits of calcium attached to the alveolar walls" in a case of renal rickets. Barr (1932), in a review of pathological calcification, mentions that cases have been recorded from the time of Virchow but states that "in human pathology the condition is extremely rare". Abnormal deposition of calcium in the tissues of the body must depend on local factors and on the metabolic regulation of calcium and phosphorus in the body fluids and skeleton. Of local conditions, healing and necrotic tissues exhibit an exceptional

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Fig 5.—Case 1   High power view of calcification in alveolar walls showing impregnation of the elastic fibres and walls of the capillaries   Von Kossa's method
×450
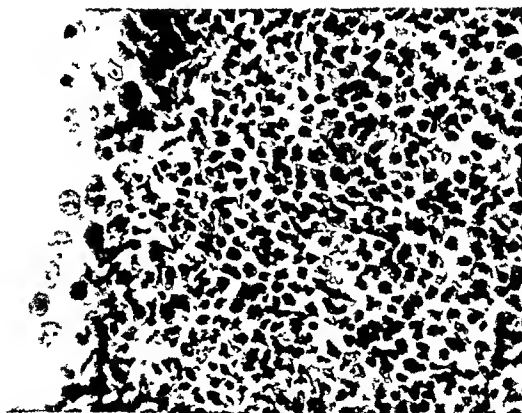
Fig 6.—Case 1   Reticulum cell sarcoma of tonsil, showing surface epithelium and underlying growth   H and E   ×450

CALCIFICATION OF THE LUNG ALVEOLI
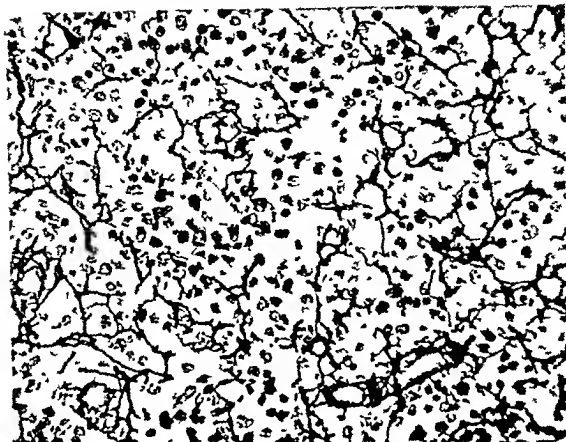
FIG 7—Case 1   Reticulum cell sarcoma of tonsil, showing reticulum impregnated by silver.   Silver stain.   ×95.

FIG 8—Case 1   Carcinoma of prostate   high power view, showing tubular cubical and columnar cell carcinoma   H. and E.   ×450

CALCIFICATION OF THE LUNG ALVEOLI
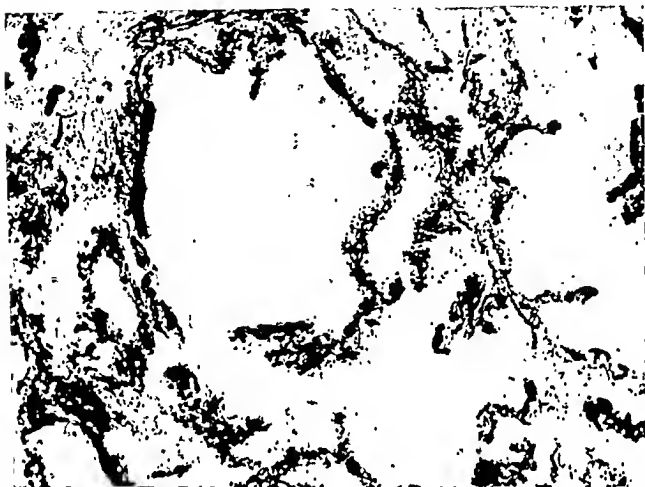
FIG. 9.—Case 2. Calcification of walls of alveoli and plaques of calcium on inner side of alveolar walls. Von Kossa's method. ×95.

FIG. 10.—Calcification of alveolar walls in partially organised pneumonia, from a boy. H. and E. ×99.

CALCIFICATION OF THE LUNG ALVEOLI
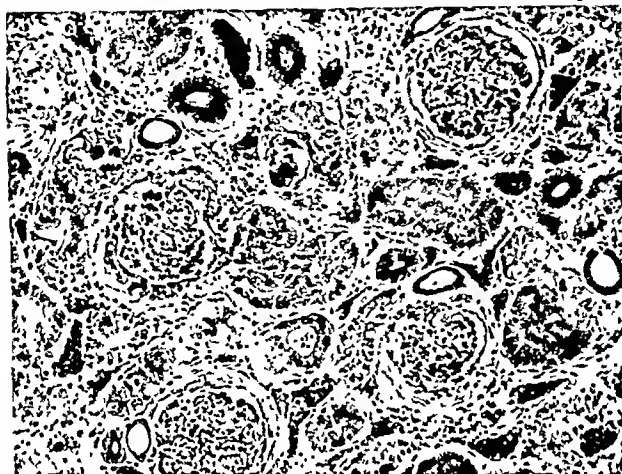
FIG. 11.—Chicken kidney, showing subacute nephritis and glomerular and tubular changes. H. and E. ×160.
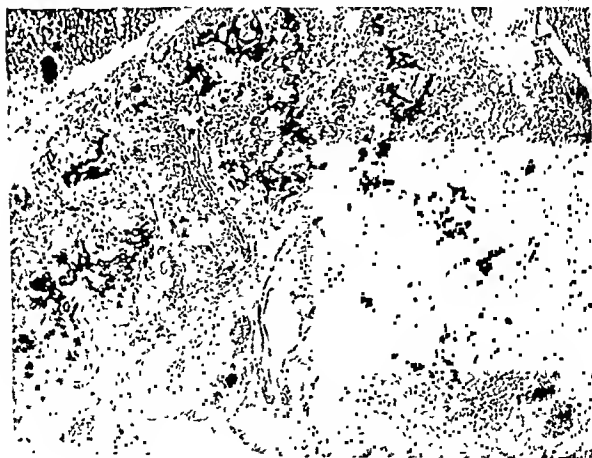
FIG 12.—Chicken lung, showing calcification of alveolar walls. Von Kossa's method. ×70

CALCIFICATION OF THE LUNG ALVEOLI
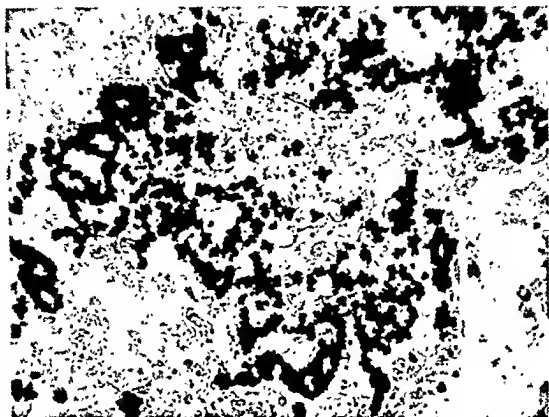
Fig. 13.—Chicken lung, showing calcification of alveolar walls. Von Kossa's method.
×290.

Fig. 14.—Chicken heart, showing calcification of myocardium. Von Kossa's method.
×70.

liability to calcification. Hueper (1927) found that experimental calcification of tissues was more likely to occur in tissues which lost acid substances, such as lungs, stomach and kidneys. It was shown by Mitchell (1930) that the *p*H of the local environment is important in maintaining the normal solubility of calcium and phosphate in tissue fluids. The discovery of phosphatases in bones led to the evolution of the Robison theory of osseous calcification. Phosphatases are not confined to the bones, and amongst other tissues they are found in the lungs. Most of the cases of metastatic calcification have occurred in renal rickets. The two cases here described were aged 62 and 48. There are no estimates available as to the degree of renal damage necessary for abnormal calcification; in neither of these cases were bony changes obvious.

SUMMARY

Two cases of severe calcification of lung alveoli are described.

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A PARAGANGLION RELATED TO THE DUCTUS ARTERIOSUS

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(PLATES CXVII AND CXVIII)

A GLAND similar in structure to that of the carotid body was found when examining serial sections of the human ductus arteriosus. This gland appears to be additional to the aortic bodies and similar glands so far described. It is figured by Boyd (1939-40) in a 28 mm. human embryo and is referred to briefly by Barnard (1939). Its position is constant between the ductus arteriosus and the pulmonary artery (fig. 1). It is always in close association with nerves, some of which appear to be large branches of the vagus (figs. 2 and 3). It also has in its neighbourhood nerve ganglion cells. It is itself very vascular and has large arteries and veins in its immediate neighbourhood. It is approximately 0.8×0.6 mm. and in some instances has smaller disconnected portions near it.

In general structure it closely resembles the carotid body, being made up of small groups of large irregularly polygonal cells with abundant protoplasm and clear-cut round or oval nuclei with a distinct chromatin net and chromatin nodes. Round the groups are spindle-shaped cells arranged in a whorled pattern and between the groups there are numerous capillaries (fig. 4). The bigger cells show occasional vacuoles and in some sections there are spaces between neighbouring cells (fig. 5). I have been unable to identify the material, if any, in these spaces. Although the carotid and aortic glands are sometimes included amongst the chromaffin system of glands they do not all appear, and this gland certainly does not appear, to show any affinity for chromic salts. In addition to the bigger nerves in the neighbourhood of the gland, nerve fibres pass freely to and can be found in the gland itself.

In addition to the carotid bodies, " paraganglia ", some chromaffin and some non-chromaffin have been described, (1) between the pulmonary artery and the left coronary artery, (2) between the arch of the aorta and the ductus arteriosus, (3) in the region of the junction of the innominate and right subclavian arteries, and (4) in the region

of the upper surface of the aorta or left subclavian artery. Many of these have been found in animals and in human embryos. Boyd (1937) in his review of the development of the human carotid body reviews also the published accounts of these "paraganglia" and their development. This is a full review with references and makes it unnecessary to go over the ground again. Boyd (p. 29) says:—"From my study of the carotid body and the structures which resemble it, I think that they are developed in relation to segments of arteries which possess a pressor-receptor apparatus; further, as was suggested by Koch (1931), the segments of the arteries which possess a pressor-receptor apparatus are persisting portions of the original embryonic branchial-arch vessels".

The functions of the gland are not known. From its structure it would appear that these would be similar to those of the carotid body, and it would be tempting to speculate as to its possible association with the closure of the ductus arteriosus. Although so far I have been unable to trace any recorded cases of tumours arising from this type of tissue in this situation, it is highly probable that it may at some time give rise to tumours in the mediastinum.

Note on fœtal fat glands

When examining fœtuses it is usual to find fat glands and unless one has seen them before they may be mistaken for other glands. They have a well defined capsule, are unusually vascular and the cells are large and filled with small or large fat droplets (figs. 6 and 7).

Summary

A gland of the carotid body and aortic type occurring at the pulmonary end of the ductus arteriosus is described.

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"   . . . . . . 1939-40. *Lond. Hosp. Gaz.*, xliii, Clin.
                                         Suppl. i, iii.

PARAGANGLION OF DUCTUS ARTERIOSUS
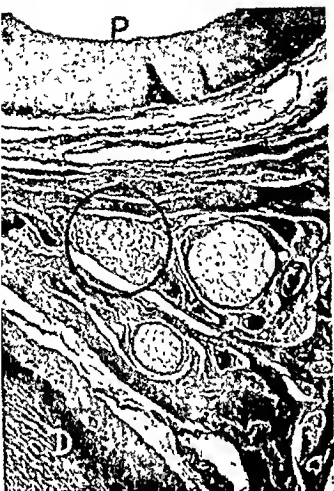
FIG. 1.—Pulmonary artery (P) and ductus arteriosus (D), showing position of gland in circle and its relation to nerves and blood vessels. Mallory's stain. ×25.

FIG. 2.—Higher-power view showing the intimate relations with nerves and blood vessels. H. and E. ×75.
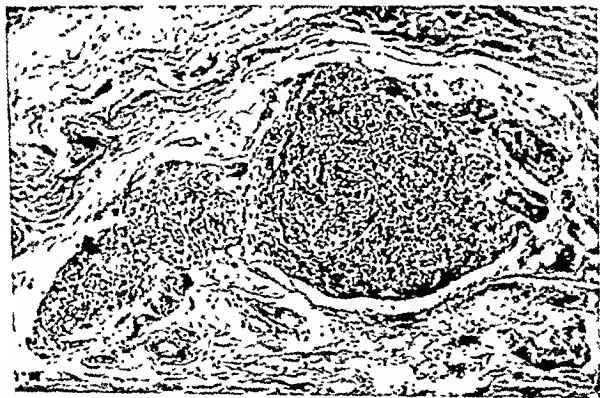
FIG. 3.—Gland showing intimate relation to nerves; in the nerve trunk are nerve ganglion cells. H. and E. ×90.

PARAGANGLION OF **DUCTUS ARTERIOSUS**
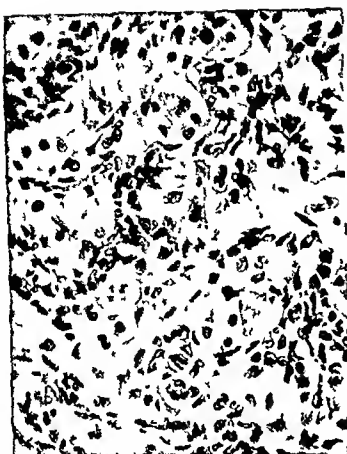
Fig 4 —High power view showing the polygonal cells and the peripheral spindle shaped cells   H and E   ×360
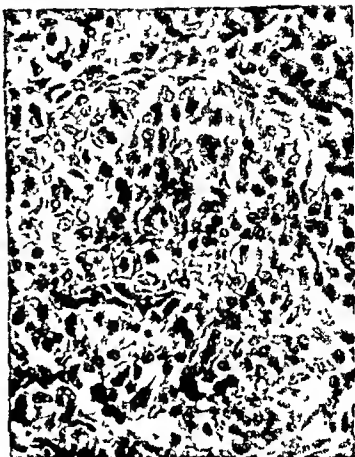
Fig 5 —High power view of another of the glands, showing vacuolation in some of the polygonal cells   H and E   ×360
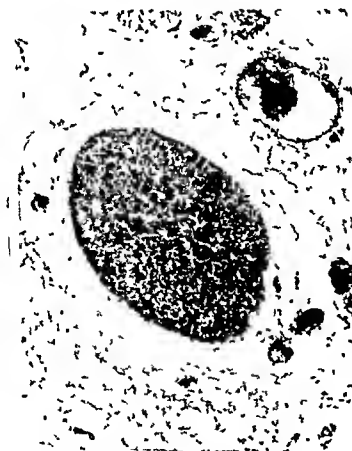
Fig 6 —Low power view of fœtal fat gland   H and E   ×75
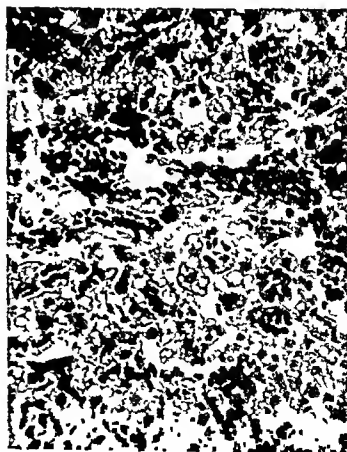
Fig 7 —High power view of fœtal fat gland, showing the large foamy fat cells and the general glandular structure   H and E   × 360

PIGMENTARY CHANGES IN FAMILIAL NON-HÆMOLYTIC JAUNDICE

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BETWEEN 1901 and 1907, Gilbert and his associates published a series of 61 papers, summarised by Gilbert *et al.* (1907), in which one of their claims was that there existed a familial disorder, which they called " Simple familial cholæmia ", characterised by a simple increase of blood bilirubin. Study of their documented cases shows that these were almost certainly cases of acholuric jaundice, and when the latter syndrome was defined, little more was heard of Gilbert's theory for many years.

Parkes Weber (1916-17) described, as a curiosity, a man who was permanently jaundiced though otherwise healthy. Gansslen *et al.* (1925) described 13 cases in which the serum gave an indirect Hijmans van den Bergh reaction and jaundice was the only symptom. Their cases, however, had increased urobilin in the urine and 10 of them had one or more relatives with acholuric jaundice.

Hijmans van den Bergh (1933) used the term " Constitutional hyperbilirubinæmia " to describe jaundice without signs of hæmolytic disease in a man aged 25 and his father, and a man aged 41 and his father, two brothers and one sister.

Rozendaal *et al.* (1935) analysed 214 cases of slight and latent jaundice and classed 48 of them as " Constitutional jaundice " owing to the absence of signs of hæmolytic disease. Four of these cases had a family history of jaundice.

Meulengracht (1939) unfortunately gave the syndrome a new name, " Icterus intermittens juvenilis ", in a description of 24 patients, all of whom complained of weakness and lack of energy and of whom 3 had a family history of jaundice. His patients were between the ages of 14 and 34.

Damashek and Singer (1941) called the syndrome " Familial non-hæmolytic jaundice " and investigated the liver function and the blood in two Jewish brothers and in an Italian family of nine, all of whom had jaundice with indirect Hijmans van den Bergh reaction and no evidence of increased hæmolysis. These authors were the first to do the crucial estimation of stercobilin excretion. They showed that it was not increased, and they considered therefore that increased hæmolysis was ruled out.

Carithers (1941) described the syndrome in four members of one family, and Curry *et al.* (1942) in a man and his sister. The man had had his spleen removed on a mistaken diagnosis of acholuric jaundice.

Malloy and Lowenstein (1940) investigated an analogous condition in a strain of rats in which jaundice was inherited as a recessive character.

The following contribution is a study of the relation between fluctuations in blood bilirubin and stercobilin excretion, in a patient who conformed to the syndrome. The absence, in his case, of a family history of jaundice is no justification for modifying the name of the disorder in the present state of our knowledge.

Case report

K. J., aged 25 years, was admitted to the Royal Hospital, Sheffield, in January 1946 for two months, for investigation of his jaundice. He was born at full term and was of a normal colour, but had some sort of respiratory spasm which changed in 24 hours to a cough, and he has had persistent and frequent cough ever since. About the 6th day he became jaundiced and this also has persisted. He was breast fed and put on weight normally. The urine was never dark. At 10 years he had a severe attack of whooping cough, and at 15 years a severe attack of influenza. The jaundice did not get worse during these infections.

As a boy he was kept indoors a great deal because of his cough and great liability to respiratory infections, but when he was able to get outside his jaundice always improved. The jaundice almost disappeared in the summer and was maximal in winter.

From 15 years to 20 years he suffered from frequent attacks of dizziness and faintness, which sometimes progressed to unconsciousness; he was sometimes unconscious for 10 minutes. These attacks were not associated with trembling or epileptiform movements. He has had frequent toothache, pains in the joints and eyes, and headache. His left arm has always been weak and subject to trembling. His appetite has been good, but he states that eggs and fats make the jaundice worse and make him feel sleepy and out of sorts. He has always felt disinclined for much effort and works as a part-time gardener.

Family history. His father and mother are English and were both born in Sheffield. Neither they nor their parents ever had jaundice. His mother is aged 66. She is well except for rheumatoid arthritis. She had weekly or fortnightly "bilious" attacks, characterised by nausea and vomiting, all her life until K. J. was born, when they ceased. Examination of her blood gave the following results: Hb 70 per cent., R.B.C. 3,600,000, C.I. 1, W.B.C. 6200. The film appeared normal. Reticulocytes were less than 0·5 per cent. Blood group A; Rh-positive. Hijmans van den Bergh reaction, direct and indirect, negative. Red-cell fragility: hæmolysis started at 0·45 per cent. and was complete at 0·35 per cent.

His father, aged 67, has always been healthy. Hb 100 per cent., reticulocytes less than 0·5 per cent. Blood group O; Rh₁rh (O).

He has two sisters, one of whom, aged 36, had a partial thyroidectomy in 1938 and was found to have achlorhydria and a congenital hour-glass constriction of the gall bladder. Since the operation she has developed a mild iron-resistant anæmia and disabling rheumatoid arthritis. Her blood bilirubin is not increased. The other sister, aged 38, has always been well except for attacks of pain down

the legs, which lasted from the time she was a baby till the age of 14. Her hæmoglobin is 100 per cent. and she has no increase of blood bilirubin.

One of the mother's sisters is said always to have had a yellow complexion.

On examination. The patient was a thin man, 5 ft. 8 in. tall and weighing 9 stone. His skin and conjunctivæ were fairly deeply jaundiced. Temperature 98·4°, pulse 64, blood pressure 134/70, respirations 20. The liver and spleen were not palpably enlarged. There was a patch of bronchial breathing at the base of the left lung. All the muscles of the left arm were weak and there was a coarse tremor of the arm when unsupported. His hands were very moist. No other abnormal physical signs. No pigmentation of the cornea.

A radiograph of the chest, combined with lipiodol injection, showed tubular bronchiectasis in the left lower lobe.

Laboratory investigations

The blood count showed Hb 100 per cent. (Haldane), R.B.C. 5,200,000, C.I. 1, W.B.C. 13,000, hæmatocrit 44, volume index 0·97, M.C.V. 84 c.µ, saturation index 1, mean corpuscular Hb concentration 32 per cent., mean diameter of red cells 7·32 µ, blood group $Rh_1Rh_1(A1)$. Numerous reticulocyte counts over a period of six months gave values that were never over 3 per 500 red cells. Red-cell fragility in hypotonic saline was tested five times in six months and was remarkably constant. Hæmolysis started at 0·475 per cent. and was complete at 0·35 per cent.—a rather high starting point for the method used, which was to suspend thrice-washed red cells in an equal volume of normal saline and to add 0·02 c.c. of this to 5 c.c. of the test saline.

The basal metabolic rate was plus 20 per cent., serum calcium was 11 mg., blood phosphorus 3·77 mg., blood phosphatase 3·37 units, blood cholesterol 170 mg. and plasma proteins 6·4 g. per 100 c.c. The Wassermann reaction was negative.

The fasting blood sugar was usually between 0·07 and 0·08 g. per 100 c.c., but on two occasions was 0·06 g. Two glucose tolerance curves each showed only a slight rise after the ingestion of 50 g. of glucose. The figures at half-hourly intervals were as follows :—

22.1.46 : Fasting B.S. 0·07, post glucose 0·09, 0·07, 0·064, 0·06, 0·058 g. per 100 c.c.

20.3.46 : Fasting B.S. 0·07, post glucose 0·08, 0·074, 0·074, 0·071, 0·075 g. per 100 c.c.

On one occasion he was kept fasting from supper time to 1 p.m. next day to see if he developed symptoms of hypoglycæmia, but his blood sugar remained constant all morning at 0·06 g.

In an insulin-sensitivity test done simultaneously with a control subject, 0·1 unit of insulin per kilo body weight was injected intravenously and capillary blood-sugar estimations were made every ten minutes. This test is also a natural adrenalin sensitivity test and

the subjective symptoms are due to adrenalin secretion. The figures (g. per 100 c.c.) were as follows :—

	Fasting	10 mins.	20 mins.	30 mins.	40 mins.	50 mins.	60 mins.
K. J. . . .	0·12	0·093	0·06	0·08	0·1	0·1	0·1
Control . .	0·116	0·1	0·08	0·085	0·087	0·09	0·1

Sweating and subjective symptoms were apparently equal in both subjects. K. J.'s pulse rate fell to 52 per minute and the control's to 64 per minute after half-an-hour.

Sternal-marrow puncture gave a normal smear and there was certainly no increase in the proportion of nucleated red cells.

Two attempts were made to secure a liver biopsy, but neither was very successful. The few liver cells present in the needle appeared normal and contained no iron pigment stainable with potassium ferrocyanide and hydrochloric acid.

The prothrombin index (method of Fullerton, 1940), was 100 per cent. In a bilirubin-excretion test, samples of serum taken three minutes and one hour after intravenous injection of 50 mg. of bilirubin both contained slightly less pigment than the pre-injection specimen. There was very little possibility of error in this result, as the sera were compared directly in the Pulfrich photometer. In the hippuric acid-synthesis test, excretion of hippuric acid was 1·23 g. in 4 hours (normal 3 g.).

Three attempts were made to pass a duodenal tube in order to study the duodenal bile, but examination with a fluorescent screen showed that the tube was held up in the much-dilated atonic stomach.

During January and February, the serum bilirubin remained about 16 mg. per 100 c.c. The direct Hijmans van den Bergh reaction was negative, the indirect strongly positive. Bilirubin was never found in the urine by Hunter's test.

The average 24-hour stercobilin excretion from two series of pooled four-day specimens, was 71 and 72 mg. respectively. Urobilin excretion was rarely over 1 mg. per day, so the total excretion was well under the normal average of 100 to 250 mg. described by Watson (1937).

Professor Rimington kindly did porphyrin determinations on four specimens of urine : all the results fell within the normal range. The average excretion in 24 hours was 37·9 γ.

A diagnosis of familial non-hæmolytic jaundice was made and in May the patient was readmitted for further studies on his pigment excretion. At that time he was only slightly jaundiced. On the day of admission a small boil on the arm was incised, and he then developed severe cellulitis of the arm and a sudden increase of jaundice. On the 19th of May he had an attack of malaise which

lasted four days and was associated with increased cough, dizziness, rapid pulse, a slight rise in temperature and a marked increase in jaundice. He said that these symptoms were very familiar to him. They were probably due to a flare-up of infection in his bronchiectatic lung.
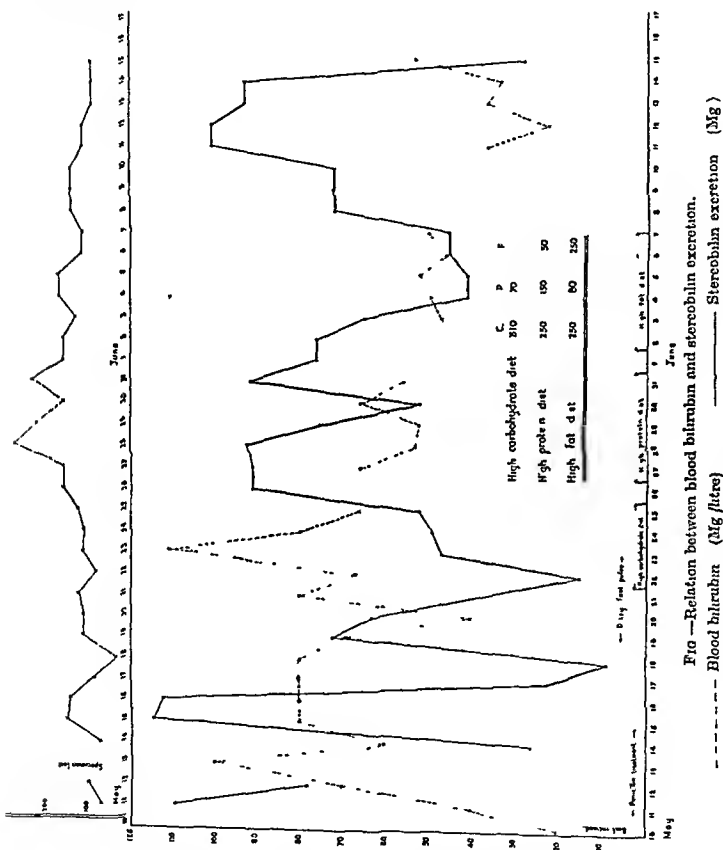
Fig.—Relation between blood bilirubin and stercobilin excretion.

-------- Blood bilirubin (Mg/litre)   ———— Stercobilin excretion (Mg)

During his stay he was given, in turn, high carbohydrate, highprotein and high-fat diets. The special diets were taken for periods of four days with intervals of two days on a normal hospital diet. The resulting changes in stercobilin excretion were difficult to assess

owing to the unstable condition of his blood bilirubin in May. The apparent increase in excretion after each test diet may have been due to the increased bulk of fæces, but the only certain conclusion that can be drawn is that the high-fat diet did not prevent or interrupt the steady fall in blood bilirubin which was then occurring, even though the amount of fat ingested was at the limit of tolerance, causing nausea and, curiously enough, itching of the skin.

The chart on p. 637 is an almost complete record of the changes in blood bilirubin and in stercobilin excretion over 34 days. Where successive stercobilin values are identical, the estimation was done on pooled specimens and then averaged for 24 hours. The urobilin excretion in the urine was estimated daily but was so slight that it has been ignored on the chart. Apart from fluctuations in stercobilin excretion between 15th and 18th May, which might have been due to variations in fæcal volume and were not associated with changes in the blood bilirubin, it can be seen that there is an apparent inverse relationship between the changes in the blood and fæces. At first sight, therefore, it looks as if the periodic increases in blood bilirubin were due to diminution in the excretion of bile pigment.

Owing to delay of fæces in the gut, however, the graph must be interpreted very differently. Charcoal as a marker was given twice during the course of the experiment and it took three days to appear in the fæces. K. J. was a good subject for this investigation, as his bowel habit was very regular. If the stercobilin excretion shown on the chart is displaced backwards three days, as it should be, the fluctuations coincide remarkably, considering the many variables. The very low pigment excretion on 18th May was largely due to the small weight of fæces passed on that day.

DISCUSSION

There are three interlinked questions in regard to the syndrome of familial non-hæmolytic jaundice which have not yet been fully answered. (1) What is the role of the liver in causing pigment retention? (2) What is the relation of the syndrome to acholuric jaundice? (3) What is the source of the increased blood bilirubin?

The role of the liver in pigment retention

The continuously subnormal stercobilin excretion shown in the chart cannot be explained by an unusually high liver threshold, for in that event, once the blood bilirubin had reached threshold level, pigment excretion would be normal.

In the active stage of jaundice, the stercobilin excretion varied directly and not inversely with the amount of bilirubin in the blood, and this makes it unlikely that the day-to-day fluctuations in bilirubin were caused by variations in liver function. A more likely explanation is that the bilirubin fluctuations were caused by daily variations in

hæmolysis such as are known to occur as a result of exercise, infections or other factors, and that the liver function was depressed to a fairly constant degree. Pigment excretion would then follow the fluctuations in blood bilirubin by the laws of mass action. It is necessary to include the clause " in the active stages of jaundice ", for I do not know what K. J.'s stercobilin excretion was when his blood bilirubin was comparatively normal. The first recorded estimation of 110 mg. on 11th May suggests that it may have been within normal limits.

The hippuric acid-synthesis test, done in January when the jaundice also was in an active phase and showing a low excretion, was further evidence of depressed liver function. It has not yet been possible to repeat the test in a quiescent phase. The jaundice increases markedly when the bronchiectasis causes increased symptoms and it is difficult to say whether this is due to increased hæmolysis or to further depression of liver function. The actual cause of defective pigment excretion is still obscure, but Malloy and Lowenstein suggested that there might be a lessened capacity to change bilirubin giving the indirect reaction into bilirubin giving the direct Hijmans van den Bergh reaction.

The relationship of the syndrome to acholuric jaundice

The early arguments over the relation of the two syndromes have been reviewed by Tecon (1938). In acholuric jaundice there are two main components, increased breakdown of red cells and defective excretion of bilirubin by the liver. The liver has a large reserve capacity for pigment excretion and, if liver deficiency is slight, increase of red-cell breakdown does not result in jaundice. Is it possible that familial non-hæmolytic jaundice is a variant, in which the liver element is supreme and the rate of breakdown of red cells only slightly increased ? It is difficult to exclude the possibility of slightly increased red-cell breakdown. The reticulocyte count is a relatively insensitive index and the same holds true of morphological changes in the red cell such as spherocytosis.

The constant low stercobilin excretion has been regarded as proof that there is no increased hæmolysis, but it has been denied that stercobilin excretion bears a quantitative relationship to blood breakdown (Rous, 1923 ; Whipple, 1926). It is possible that blood bilirubin is deviated elsewhere or excreted elsewhere than in the bile. Evidence suggesting deviation of blood bilirubin was given by Vaughan (1942), who found that the rise in serum bilirubin following transfusion of stored blood was not associated with a significant increase of stercobilin excretion. She thought that the pigment might have been retained for hæmoglobin regeneration. Similar discrepancies between blood and bile pigments in dogs with biliary fistulæ were described by Broun *et al.* (1923).

A study of the quantitative changes in the blood bilirubin shown in the chart suggests that it is not necessary to postulate increased hæmolysis, for the highest rise in 24 hours was 4·6 mg. per 100 c.c. This is equivalent to a total increase in the blood serum of 145 mg. Hæmatin and bilirubin are roughly equivalent in weight and, as hæmoglobin yields 4 per cent. by weight of hæmatin, 145 mg. of bilirubin are equivalent to about 3·61 g. of hæmoglobin. Three or four times this amount is probably liberated every day in normal people. The evidence, therefore, suggests that increased hæmolysis is absent in familial non-hæmolytic jaundice and that therefore the two syndromes are essentially different.

The source of the increased blood bilirubin

Though the fluctuations in jaundice can be explained on the basis of the normal daily fluctuations in hæmolysis, it has never been proved that bilirubin is solely derived from the breakdown of red cells. In the case reported, the muscular weakness and tremor in the left arm and the relationship of fluctuations in the jaundice to exercise, raised the intriguing speculation that the disorder might be a familial muscular dystrophy and the blood bilirubin derived from myohæmoglobin. Meulengracht stressed the uniform complaint of tiredness and lack of energy in familial non-hæmolytic jaundice, and the tendency for the jaundice to improve with age. Whipple found that the myohæmoglobin content of the muscles of dogs is increased with age and the activity of the animals. On the other hand, myohæmoglobin is rapidly excreted by the kidneys when it gains access to the blood stream and it is hard to see that much of it could be transformed into bilirubin by the reticulo-endothelial system. K. J.'s excretion of creatinine in the urine was estimated for a week, during an active phase of jaundice, and was nearly constant at 1·5 g. per day. There was no creatinuria.

SUMMARY

1. A case of familial non-hæmolytic jaundice is reported in which stercobilin excretion was persistently subnormal over a period of 34 days.

2. The relationship of stercobilin excretion to changes in the blood bilirubin in this case suggested that there was a constant defect in the excretion of bile by the liver during the phase of active jaundice.

3. The maximum daily fluctuations in blood bilirubin could be explained quantitatively by normal variations in hæmolysis.

4. The relationship between this syndrome and acholuric jaundice is discussed.

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GLIOMATA OF THE NOSE AND ORAL CAVITY: A REPORT OF TWO CASES

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(PLATES CXIX-CXXII)

THE rarity of tumours of neuroglial tissue in the nasal and oral regions warrants the publication of two additional cases.

Case I

Clinical history. A female infant, born in Hackney Hospital by normal labour at full term and then weighing 6 lb. 14 oz. The mother was aged 25 years; it was her first pregnancy and there was no abnormality during it. The baby cried at once and showed no signs of asphyxia. A mass was noticed in the palate displacing the uvula to the left and also palpable in the right maxillary region. This caused severe dysphagia from the first; by the second day it had grown rapidly and there was respiratory stridor. The infant died 3 days after birth.

Summary of necropsy

Inanition. Neuroglial tumour of soft palate. Milky material in larynx and trachea. Slight bronchopneumonia and congestion of lungs. No abnormality of heart or vessels. Cloudy swelling and œdema of liver and kidneys. Œdema of spleen. Mucous catarrh of stomach. Œdema of brain. Middle ears clean. No abnormality in suprarenals, pancreas, intestines, urinary bladder, uterus or ovaries. Slight jaundice and cyanosis.

Projecting into the right side of the oral cavity was a mass 3·2 cm. from side to side by 2·4 cm. from before backwards and 1·0 cm. high (fig. 1). It extended for a distance of up to 0·5 cm. to the left of the middle line. Anteriorly it appeared, at its base, co-terminous with the posterior margin of the hard palate; a rounded free surface projected anteriorly beyond this for a distance of about 0·3 cm. The postero-inferior border lay just above the right faucial tonsil. The right border was ill-defined and extended into the sub-mucous tissues of the cheek. The surface was irregularly rounded and for the most part had a smooth, purplish grey covering resembling buccal mucosa; the surface of the most prominent central part was greyish yellow and slightly granular.

On section the total thickness of the mass was 1·5 cm. and the deep aspect was not clearly demarcated. The greater part of the cut surface consisted of homogeneous greyish white tissue with faint ill-defined flecks and streaks of grey. The left border was occupied by a group of cysts up to 1·2×0·7×0·6 cm., with a smooth, greyish white lining. Just external to these was a cleft, 0·4 cm. long by 0·05 cm. wide, with a blackish brown lining and with a dark brown fleck close to one end. External to this and close to the projecting surface were a few ill-defined greyish brown areas up to 0·5×0·25 cm. Near the extreme right margin there were a few cysts up to 0·25 cm. diameter with smooth grey linings.

Histology

The specimen was fixed in 4 per cent. formol-saline and later transferred to Kaiserling I solution. Representative portions from the tumour were embedded in paraffin and sections were stained with Ehrlich's hæmatoxylin and eosin, iron hæmatoxylin and van Gieson's stain, Weigert's elastic stain and Mallory's phosphotungstic acid-hæmatoxylin.

On microscopical examination the most abundant and conspicuous element is a tissue consisting of a feltwork of fine fibrils which stain pale purple with Mallory's phosphotungstic acid-hæmatoxylin. Among the fibrils are cells with fibrillary processes and usually scanty protoplasm and an oval or round nucleus occasionally showing a nucleolus. This tissue histologically resembles neuroglia and the cells appear for the most part to be of the nature of pilocytic astrocytes, though there are also a few small round cells and occasionally more swollen cells which suggest oligodendroglia. There are large ill-defined areas (fig. 2) in which the meshes of the feltwork are wide and tend to form long, roughly parallel clefts; considerable numbers of fine collagen fibres run through these areas parallel with the clefts, usually singly, but rarely in small bundles of two to four fibres. In other large areas there are wide bands and small sheets of dense fibrous tissue in which lie islands of glial tissue; in these islands the feltwork is close-meshed, showing only a few irregular clefts, the cells have a more irregular shape and more cytoplasm and collagen fibres are absent. Towards the outer border of the mass is a considerable amount of voluntary muscle, comparable in amount and on the whole in arrangement with that present in the soft palate of infants of similar age. In one area, however, the muscle bundles are separated by wide bands of fibrous tissue, by lobules of adipose tissue and by irregular areas and bands of glial tissue. The feltwork is here close-meshed and the cells have more rounded bodies and abundant cytoplasm. The tumour tissue is occasionally so intimately mingled with the muscle that groups of short lengths of one to four muscle fibres, retaining their cross striation, may be seen completely surrounded by glia (fig. 3).

Near the buccal surface some of the islands of glia have central

PLATE CXIX

GLIOMAS OF NOSE AND ORAL CAVITY

FIG. 1.—Case I   Tumour of soft palate   ×2 circa

GLIOMAS OF NOSE AND ORAL CAVITY
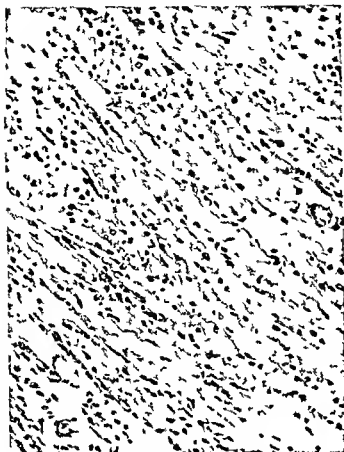
FIG 2 —Case I Area of loose meshed glio matous tissue containing collagen fibres Hæmatoxylin and eosin ×100
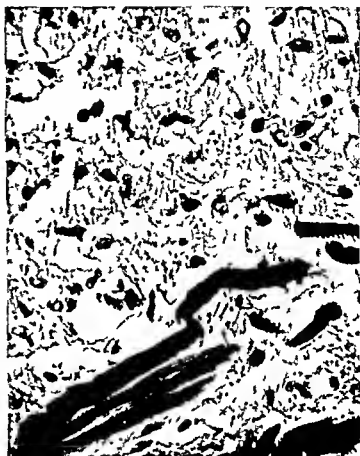
FIG 3 —Case I Voluntary muscle fibres lying in gliomatous tissue Mallory's phosphotungstic acid hæmatoxylin ×440

FIG 4 —Case I Clefts in island of glial tissue and tubules lined with ependymal cells Mallory's P T A H ×115

FIG 5 —Case I Immature choroid plexus H and E ×160

clefts lined wholly or in part with low-columnar or cuboidal cells (fig. 4), in a very few of which small groups of blepharoplasts are present. In this area there are also a few small cysts or tubules lined with similar cells lying directly on a collagenous wall.

In the outer part of the mass there is a fibrous band containing a number of small spaces into which project small vascular collagenous papillary processes. Low-columnar cells line these spaces and cover the papillæ, which bear a strong resemblance to those of choroid plexus (fig. 5); a very few of the covering cells contain small groups of blepharoplasts.

The cleft with a blackish-brown lining observed naked eye is seen to be lined on one side with a layer of cells loaded with blackish-brown pigment which contains no free . on and gives a positive reaction for melanin with Fontana's stain. The opposite side is lined with a zone of cells eight to twelve cells thick, which are for the most part rounded and of primitive type but which, at a point where the two sides of the cleft come into apposition, are more elongated at right angles to the surface and suggest differentiation into rods and cones (fig. 6). The dark-brown fleck is seen to be a small cleft whose opposite sides are lined respectively with the same two types of tissue as the large cleft, with which it may well be continuous out of the plane of the section. Cords of primitive tissue are also present in other sections from this region (fig. 7).

The walls of the large cysts at the inner end of the mass consist of collagen. Some are lined with stratified columnar epithelium not unlike that of palatal mucous glands; in others the lining is flattened and resembles endothelium. The greyish-brown areas just external to the cleft with blackish-brown lining consist of fibrous tissue containing numerous small vessels, some of which lack an external elastic lamina, though the arrangement of the fibrous tissue does not suggest pia mater. Near the voluntary muscle is a small island of cartilage, similar to some present in the apparently normal soft palate of an infant of similar age. A few mucous glands are also present. The free surface of the tumour is covered by stratified squamous epithelium of buccal type, replaced in the most prominent central part by a fibrino-purulent layer of superficial ulceration.

Case II

Clinical history. A female infant aged 1 month was admitted to St Bartholomew's Hospital, Rochester, with a tumour of the skin of the side of the nose. This was removed surgically.

The specimen was a dome-shaped nodule $2 \times 1 \cdot 5$ cm. $\times 1$ cm. high. The upper and lateral aspects were covered with smooth white skin, apparently normal except for operative lacerations. The cut surfaces of vertical slices showed grey moist gelatinous tissue. The lower surface of removal was lacerated but appeared to consist of the gelatinous tissue cut through.

Histology

Three blocks of the tissue were embedded in paraffin. Sections were cut and stained with Ehrlich's hæmatoxylin and eosin, iron hæmatoxylin and van Gieson's stain and Mallory's phosphotungstic acid-hæmatoxylin.

On microscopical examination the dermis is seen to be occupied by tissue having the characters of neuroglia (fig. 8). There is a felt-work of fine pale purple fibres proceeding from cells of which some have scanty cytoplasm and oval or round nuclei and resemble fibrillary astrocytes, while others have large swollen bodies with one or more nuclei and appear to be gemistocytic astrocytes (fig. 9). This neuroglial tissue is traversed by collagenous strands containing blood vessels. It often surrounds the appendages of the skin but does not appear to reach the epidermis (fig. 10).

DISCUSSION

These two tumours are composed principally, if not entirely, of derivatives of medullary epithelium. The nasal tumour from case II is entirely of this origin and differentiation has taken place wholly into astrocytic neuroglial tissue, so that the tumour may well be characterised as a glioma of astrocytoma type.

The bulk of the palatal tumour from case I is also neuroglial, the cells being almost all astrocytes, but cords of primitive cells suggesting medullary epithelium are also present and, in addition, differentiation has taken place into ependymal and allied structures. The presence of blepharoplasts in a few of the cuboidal cells lining clefts in the neuroglial islands or neighbouring tubules confirms the view that these cells are ependymal in nature. The epithelium of the choroid plexuses is ependymal in origin and possesses cilia and blepharoplasts between the 7th and 22nd weeks of intra-uterine life (Russell, 1939); though no cilia are present on the epithelium covering the papillary processes described above, the presence of blepharoplasts in a few of the cells shows that they are in fact ependymal and the processes must therefore be considered as primitive choroid plexus. The rods and cones of the retina are homologous with the ependyma, and the primitive cells lining one side of the blackish brown cleft show differentiation in the direction of these structures at the point where they approach nearest to the pigmented layer lining the opposite side of the cleft. This layer consists of cells loaded with melanin and resembles the pigmented layer of the retina, a structure derived from the outer layer of the optic cup (Hamilton, Boyd and Mossman, 1945). Thus this tumour shows primitive tissue suggesting medullary epithelium and differentiation both into neuroglial tissue of astrocytomatous type and towards ependyma, choroid plexus and retina.

Glial tumours of the nose and oral cavity have been described by

GLIOMAS OF NOSE AND ORAL CAVITY

FIG 6 —Case I   Cleft lined on one side with
a layer of pigmented cells and on the other
with primitive cells partly modified in the
direction of rods and cones   H and E
×47

FIG 7 —Case I   Cords and sheets of primitive
cells   H and E   ×180

FIG 8 —Case II   Astrocytomatous tissue in tumour of nose   H and E   ×180

PLATE CXXII

GLIOMAS OF NOSE AND ORAL CAVITY
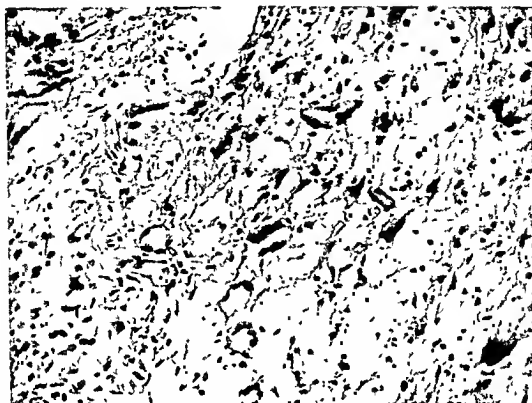
FIG. 9.—Case II. Multinucleate astrocytes of gemistocytic type. Mallory's P.T.A.H.
× 180.

FIG. 10.—Case II. Neuroglial tissue surrounding skin appendages. Mallory's P.T.A.H.
× 140.

Schmidt (1900), Payson Clark (two cases) (1905), Süssenguth (1909), Anglade and Philip (1920), Berblinger (1920-21), Rocher and Anglade (five cases) (1924), Terplan and Rudofsky (two cases) (1926), Guthrie and Dott (1927), Tobeck (1929) and Eigler (1937). The tumour in Terplan and Rudofsky's second case was situated in the tongue and is described as a neurinoma. In all the other cases the tumour was in the nose, some being external and some internal. Rocher and Anglade's second case, which communicated with the interior of the skull, had a central cavity resembling an ependymal canal, and their third case showed a few nerve cells of pyramidal type ; Guthrie and Dott did not consider the glial tissue composing the centre of their tumour to be neoplastic. With these exceptions all the tumours are described as consisting of gliomatous tissue with a varying admixture of collagen fibres and often numerous vessels, and the tumour in our case II falls well into line with them. Our palatal tumour, though mainly consisting of gliomatous tissue, shows much greater variation in differentiation and we have not been able to trace any similar tumour in this site.

Various authors have discussed at length the origin of the neurogenic tissue from which these tumours are derived. Schmidt decided that his case was not a teratoma because it consisted entirely of neuroglia and he did not know of an example of a teratoma in which only glial tissue had developed ; this argument has since been reinforced by the occurrence of further purely glial tumours in this site. He came to the conclusion that his tumour was derived from an encephalocele which had become cut off from its origin ; Guthrie and Dott attributed a similar derivation to theirs and Süssenguth's conclusions were not dissimilar. Nerve cells might well be expected to be present in an encephalocele but have not been found except in Rocher and Anglade's third case, and Berblinger pointed out that a true encephalocele should present itself in the neighbourhood of the fronto-nasal process ; these tumours however may be found anywhere in the nasal region.

Payson Clark attributed his tumours to an extra-cranial separation of embryonal neuroglia. Berblinger considered that his arose from tissue with only limited powers of differentiation cut off in early intra-uterine life ; he was the first to stress the blastomatous nature of the tumours and to apply to them the term " choristoma " in the sense of a blastomatous growth of embryonally separated primitive tissue. Later authors have been basically in agreement with him.

In our opinion this concept is applicable to the origin of our nasal tumour, the origin of which may well be accounted for in terms of displacement of marginal islands of tissue from the optic plate or of the adjacent pluripotent neural ectoderm, which remained in the surface ectoderm and were not invaginated during the closure of the neural tube and the formation of the optic cup. The origin of our palatal tumour presents greater difficulty, as the above concept

cannot readily be extended to account for it. On embryological grounds it should probably be regarded as a teratoma, but the histological picture does not altogether support this, as the tumour consists almost entirely of neuroglial and allied tissue. Such other structures as are present, embedded as they often are in the glial tissue like the appendages of the skin in the nasal tumour, can all be interpreted as remnants, some of them rather modified, of the normal constituents of the soft palate.

SUMMARY

1. Two tumours of neuroglial tissue are described, situated respectively in the soft palate and in the skin of the side of the nose.

2. The degree of differentiation in the two tumours is compared. The palatal tumour shows primitive tissue suggesting medullary epithelium and differentiation into neuroglial tissue of astrocytomatous type and towards ependyma, choroid plexus and retina. The nasal tumour is composed wholly of astrocytomatous neuroglial tissue.

3. The origin of these and similar tumours previously reported is discussed. The nasal tumour is considered to be a blastomatous growth of embryonally separated primitive tissue but this concept is inadequate to explain the palatal tumour.

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616 . 133 . 33 + 616 . 145 . 13]—002 : 616 . 831 . 9—002
(Pneumococcus)

CEREBRAL ARTERITIS AND PHLEBITIS IN PNEUMOCOCCAL MENINGITIS

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(PLATES CXXIII-CXXVI)

HITHERTO pneumococcal meningitis has usually proved a rapidly fatal disease, but it is now established that adequate treatment with penicillin will control the infection, with very great reduction in mortality (Smith, Duthie and Cairns, 1946). Failure may follow the withholding of penicillin at early stages of the infection or the loculation of pus at some point in the cerebrospinal pathway at later stages. Such collections may obstruct the circulation of the fluid, or serve as sources of reinfection with relapse if treatment is stopped too soon.

Some of the fatal cases in which death has been delayed by chemotherapy show the progressive alterations in the cerebral blood vessels described below. These are of considerable interest, clinically because they explain certain neurological signs which may appear during treatment of fulminating or protracted cases, pathologically on account of their close resemblance, in the more protracted infections, to those of polyarteritis nodosa. While our main description in what follows will concern these more advanced lesions, we propose to deal also with the vascular changes that may be detected in the early stages of the inflammatory process, since it is probable that the later vascular changes evolve from the earlier.

CASE REPORTS

I. *Early or acute stage*

Case 1. Fulminating pneumococcal meningitis (pneumococcus type 18)

· M. S. (R.I. 19095/43), aged 3 months, the second of twins, died 36-48 hours after the onset of clinical signs of meningitis. One week before admission he developed a cold and running nose from which he appeared to recover completely. On the morning of admission he was pale and did not take his

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feeds; he slept throughout the day and his cry became weak. At 6 p.m. he began to twitch. At 10 p.m., on admission, he was unconscious, pale and wasted, with a muco-purulent discharge from his mouth, nose and ears (pneumococci on culture). At frequent intervals he had convulsions, with tonic deviation of his eyes to the right, followed by a scream and clonic movements of his limbs, mainly on the right side. He was given sulphonamides systemically and one intraventricular injection of penicillin, but he died 16 hours after admission.

Lumbar C.S.F.—pressure 255 mm.; white cells 150 (mainly polymorphonuclears); total protein 250, chlorides 695 mg. per 100 c.c.; sugar absent; films and cultures showed profuse pneumococci. The ventricular cerebrospinal fluid gave almost identical findings.

Necropsy (P.M. 493. 1943). No significant changes were found apart from the brain. The right middle ear contained pus, and cultures from both middle ears grew pneumococcus type 18.

The cerebral hemispheres appeared full and soft, and the leptomeninges contained an uneven profuse yellowish-green exudate which was more abundant over the convexities than the base and was particularly dense in the sulci bordering the superficial veins. Some of these were distended with purple thrombus. On section the occipital horns were slightly dilated, especially the left, but the ventricles were otherwise unaltered, the ependyma being smooth and glistening. The choroid plexuses were moist and pale. There was slight injection of the white matter about a needle track in the left frontal lobe. An ill-defined patch of pinkish-grey softening occupied the white matter lateral to the left occipital horn.

Microscopic examination of brain. In the left frontal and occipital lobes there is intense purulent leptomeningitis with deposition of much fibrin, especially about some of the larger veins. These show purulent phlebitis and occlusion by unorganised ante-mortem thrombus in which occasional Gram-positive cocci are present. Vast numbers of these cocci swarm in the leptomeninges. There is swelling and, in places, early proliferation of the endothelial cells in some of the meningeal arteries. In the adjacent cortex there is severe œdema and degeneration, approaching necrosis in places, associated frequently with a diffuse infiltration with degenerating neutrophil leucocytes (figs. 1 and 2) and, in the occipital lobe, large foamy amœboid cells. The purulent infiltration extends down the perivascular sheaths of the perforating vessels. In general there are few cocci in the sheaths and none in the adjacent cortical tissue.

In the white matter there is patchy œdema and, in the left occipital lobe, necrosis adjacent to the ventricle. The ependyma has been destroyed in some areas, and here the subependymal glia, and in particular the sheaths of the vessels, which are cuffed with leucocytes and mononuclear cells, contain groups of cocci. Elsewhere many organisms lie on the surface of the ependyma. They also penetrate into the œdematous tissues bordering the needle track in the frontal lobe.

*The choroid plexus of the lateral ventricle shows great leucocytic

ARTERITIS IN PNEUMOCOCCAL MENINGITIS
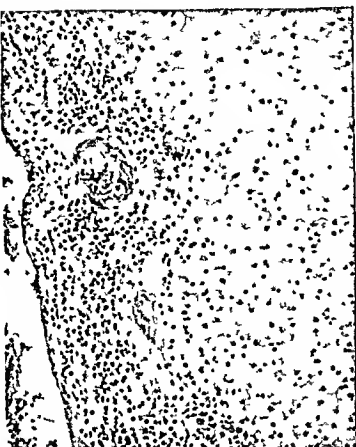
FIG 1—Case 1 Cerebral convexity, to show meningitis and diffuse infiltration of subjacent cortex H and E   ×130
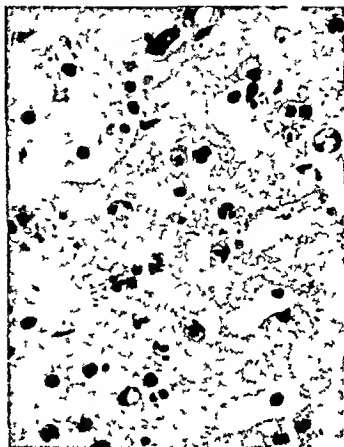
FIG 2—As fig 1, to show, at higher magnification, leucocytic infiltration of cortex H and E   ×350

FIG 3—Case 1 Choroid plexus, showing leucocytic infiltration of stroma of a villus H and E   ×500

FIG 4—Case 4 Artery in leptomeninges over medulla, showing deposits of fibrinoid material (black) in two places in swollen intima Phosphotungstic acid hæmatoxylin   ×180

infiltration of the stroma of the tips of the villi (fig. 3) unaccompanied by cocci. Many diplococci and leucocytes lie outside the plexus in the folds between the villi. The epithelium is for the most part intact but the cells often show severe pyknosis.

About the fourth ventricle and medulla oblongata there is a similar meningitis and inflammation of the choroid plexus. Numerous cocci are present in the meninges and penetrate into the neural tissue beneath the ependyma of the lateral recesses.

Case 2. Fulminating pneumococcal meningitis (pneumococcus type 18)

T. F. (R.I. 20068/43), a female aged 21, who was a semi-invalid from heart disease, developed a bad cold followed next day by headache, vomiting and incontinence of urine and fæces. Later she became unconscious and she died on the fifth day after the onset of headache, 3 hours after admission to hospital.

Lumbar C.S.F.—40 white cells per c.mm., mostly polymorphonuclears; total protein 250, chlorides 695 mg. per 100 c.c. Films and cultures showed abundant pneumococci and blood culture also gave a very heavy growth (type 18).

Necropsy (P.M. 537. 1943). Severe mitral stenosis from chronic rheumatic endocarditis. No evidence of active endocarditis. Induration of lungs. Slight back-pressure congestion of liver and of spleen. Remaining organs apart from brain normal.

The cerebral hemispheres were full and the leptomeninges greatly engorged. The sulci over the convexities were filled with yellowish-grey pus, diminishing in intensity towards the occipital poles. A considerable amount of exudate was also present over the upper part of the vermis and in the Sylvian fissures, but was scanty over the brain stem. There were a few recent hæmorrhages, up to 1·5 cm. in diameter, in the meninges over the temporal lobes. On section the brain was greatly congested, the ventricles small and the ependyma of normal appearance. Apart from congestion the choroid plexuses appeared normal.

Microscopical examination. There is intense purulent leptomeningitis, similar to that in case 1, over the cerebral convexities, the medulla oblongata and cerebellum. Slight purulent thrombophlebitis is present in a few of the larger superficial veins, and, rarely, raising of the arterial endothelium by a crescentic mass of neutrophil leucocytes. Acute purulent panarteritis affects the perforating arteries of the right frontal lobe, accompanied by hæmorrhage and purulent infiltration of the perivascular sheaths, especially in the subcortical white matter. This is often associated with irregular areas of severe degeneration and necrosis of the adjacent white matter. Great numbers of Gram-positive cocci are present in the sheaths, but none in the nervous tissues.

The ependyma of the lateral ventricles and the choroid plexuses of the lateral and fourth ventricles are overlaid with groups of

neutrophil leucocytes mixed with red corpuscles and large mononuclear cells containing Gram-positive cocci. The choroid plexuses of the lateral ventricles however appear normal, but that of the fourth ventricle shows aggregates of neutrophil leucocytes in the tips of the villi.

These two cases are characteristic examples of the fulminating type of infection with death in 2 and 5 days from the onset and without adequate chemotherapy. It is instructive to compare them with the following example, in which effective chemotherapy was in progress and death was due to fat embolism.

Case 3.   Compound fracture of anterior cranial fossa.   Pneumococcal meningitis treated with penicillin.   Fat embolism.   Death

L. L. (R.I. 35174/44), aged 32. Head injury with fractured base. Compound fracture of tibia and fibula. Twenty-four hours after the accident he became irrational and was given sulphonamides. Two days later pus and pneumococci were found in his C.S.F. and he was given penicillin intrathecally. He died of cerebral fat embolism on the third day of penicillin therapy, by which time, though scanty cocci were still seen in the films, cultures had become sterile and the cells (450 per c.mm.) and protein (100 mg. per 100 c.c.) were falling.

The meningitis microscopically shows evidence of resolution. Thus it is patchy over the central convexity and medulla oblongata, and most of the neutrophil leucocytes are in stages of necrosis. They are mixed with a good many large mononuclear cells some of which contain leucocytes. No organisms were demonstrated. Occasional focal deposits of fibrin are present in the arachnoid membrane. There are no changes in the walls of the blood vessels either of the leptomeninges or of the cortex. In the ependyma of an occipital horn the epithelium is missing in many places and is coated in some areas with a layer of pus which is infiltrated, from the ependymal side, with large mononuclear cells. Many Gram-positive cocci are present here, the majority being intracellular. A few small hæmorrhages, sometimes ring-shaped, occupy the subependymal tissues, which are œdematous and sparsely infiltrated with large mononuclear cells and occasional neutrophil leucocytes. Many of the vessels are cuffed by similar cells. No changes were identified in the walls of the vessels beyond fibrinoid necrosis in a single example which lay at the centre of a ring hæmorrhage.

Comment on early stage

Examination has shown that there is a wide distribution of the infection throughout the ventricular system as well as the meninges at this early stage. The ependymitis and leucocytic infiltration of the choroid plexuses are interpreted as due to reflux of infected fluid from the basal meninges. Alternatively it might be argued that infection reached the brain through the choroid plexus, except in

case 3, where a fracture of the ethmoid sinus with an overlying dural tear was responsible for the meningitis. But the infiltration of the plexus of the fourth ventricle in case 2, while those of the lateral ventricles were unaffected, argues in favour of a retrograde spread of the infection.

Purulent arteritis and phlebitis with thrombosis were observed in the meningeal and perforating vessels in cases 1 and 2, with associated severe degeneration and necrosis of the neighbouring brain substance. Occasionally Gram-positive cocci had invaded the thrombus in the veins. Early endarteritis was found in a few otherwise unaffected meningeal arteries in cases 1 and 2. That these vascular changes are due to the pneumococcal infection and not to any chemotherapeutic measures is proved by their presence in 14 of 28 cases of pneumococcal meningitis which came to necropsy at the London Hospital in early stages of infection before 1940. Moreover in 3 cases of the latter series there is conspicuous fibrinoid necrosis of occasional perforating arterioles in the cerebrum.

II. *Intermediate stage*

Case 4. Relapsing pneumococcal meningitis associated with vascular hypertension

R. R. (R.I. 24155/44), male, aged 50. Pneumococcal meningitis (pneumococcus type 8) of about 18 days' duration, beginning with pain in left ear. Penicillin treatment begun on eleventh day of illness and stopped four days later, whereupon meningitis recurred and the patient died in spite of resumption of penicillin (Smith *et al.*, case 5). At the time of the relapse his blood pressure, which had previously been 130/80, rose to 230/130 and remained high until a few hours before death. Rapid pulse, persistent subnormal temperature, profuse sweating and hæmatemesis for several hours before death.

Necropsy. (*P.M.* 135. 1944). Both middle ears contained mucopus. The other accessory sinuses were empty, apart from the sphenoidal which contained brown-stained mucus.

The convexities of the brain were dry and greatly congested. Purulent meningitis was localised to an irregular band of thick exudate along the parasagittal borders of the frontal lobes and over the base, where it obscured the vessels and nerve roots, extending into the Sylvian fissures and filling the basal cisterns. The cisterna magna however contained little pus and was small, being encroached upon by the cerebellar tonsils which had herniated into the foramen magnum to a depth of 1·1 cm.

On section the substance of the brain was deeply congested. There was moderate symmetrical dilatation of the lateral ventricles and considerable expansion of the anterior part of the third. The fourth ventricle was small. The ependyma was coated with purulent exudate forming in places a well defined, lightly adherent membrane which became continuous throughout the third ventricle and formed

a stalk plugging the whole length of the aqueduct. Purulent exudate also coated the choroid plexuses.

The meninges of the spinal cord contained an uneven layer of purulent exudate over the posterior surface except for a length of 13 cm., the site of lumbar injections of penicillin over the upper part of the cauda equina, where they were translucent but contained an elongated mass, 7×1 cm., of recent blood-clot. The terminal part of the cauda equina was filled with thick pus. On section the cord was injected but otherwise normal.

Microscopical examination. The parasagittal region of the right frontal lobe, the medulla oblongata and an upper thoracic and a lumbar segment of the spinal cord show much necrotic pus beneath the arachnoid membrane. A zone of tangled threads of fibrin separates this from the pia, in which collagenous thickening is associated with a cellular infiltration, mainly of lymphocytes and plasma cells, extending into the sulci and, in the cord, infiltrating the posterior roots. Organisms are demonstrated only in the frontal region where a few feebly Gram-positive cocci occur in the old pus.

Many of the larger veins over the frontal lobe contain recent unorganised thrombus, occasionally spreading into the perforating veins of the cortex ; this is associated with a few subcortical areas of recent hæmorrhage and softening infiltrated sparsely with neutrophil leucocytes. Focal fibrinoid necrosis affects the media of many arteries and arterioles in the meninges over the medulla oblongata. In others the intima is focally thickened by œdema, neutrophil leucocytes and plaques of fibrin (fig. 4). Similar changes are observed in the meningeal vessels of the spinal cord. In one arteriole a small collection of foam cells is present beneath the endothelium.

The ependyma of the right lateral ventricle is destroyed in places and is overlaid by fibrin and pus. The residual ependymal cells are devoid of cilia and their nuclei are pyknotic. The subependymal glia is œdematous and most of the vessels have undergone fibrinoid necrosis (fig. 5). In general there is little associated perivascular cellular infiltration, but some of the larger veins are cuffed with small lymphocytes. The intervening glia is sparsely infiltrated with lymphocytes and disintegrating leucocytes. Small hæmorrhages are also present. No organisms are demonstrated.

The choroid plexuses of the left lateral and fourth ventricles show great leucocytic infiltration of the stroma at the tips of the villi. The epithelium is for the most part intact but there is focal degeneration of the cells, with karyolysis and hyaline-droplet degeneration. Much pus is present in the interstices between the villi. No organisms are demonstrated.

In another case of relapse following premature stopping of penicillin treatment and death on the 13th day of meningitis the pathological findings in the central nervous system are almost identical and the vascular changes are similar. Thus in the meninges

ARTERITIS IN PNEUMOCOCCAL MENINGITIS
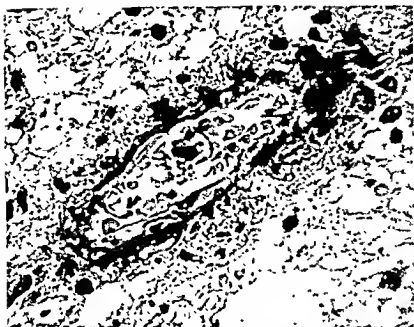
FIG. 5.—Case 4. Subependymal arteriole from lateral ventricle, showing fibrinoid necrosis. H. and E. ×560.

FIG. 6.—Case 4. Pial arteriole from cervical cord, showing necrosis and purulent infiltration of media in left upper border, and raising of endothelium by red corpuscles and a few leucocytes. H. and E. ×180.

FIG. 7.—Case 5. Coronal section of brain, showing collapsed subdural abscess skirting medial and ventral aspect of right cerebrum.

over the right frontal lobe a few vessels show purulent arteritis and others fibrinoid necrosis; they coincide with points of maximal cellular infiltration of the meninges. Beneath the ependyma a few small arterioles have undergone fibrinoid necrosis and over the brain stem some arteries show focal purulent infiltration of the intima with the deposition of fibrin, or complete zones of fibrin throughout the intima. The adventitia is often infiltrated with mononuclear cells and neutrophil leucocytes and, in places, these cells extend into the media, with destruction of the muscle and deposition of fibrin. Arteries of all calibres are affected. Similar focal purulent arteritis or fibrinoid arteriolar necrosis appear in the pia of the cervical cord (fig. 6). The substance of the cord is unchanged.

Comment on cases in intermediate stage

In these two cases the meningitis had entered a more chronic phase, accompanied by stagnation of the pus in different parts of the cerebro-spinal pathway and pyocephalus. The vascular changes in the meninges and nervous tissues were of much the same character as in the early stage, but were almost entirely restricted to arteries. Purulent infiltration of the whole thickness of the wall or of the intima alone, and fibrinoid necrosis either of the whole wall or of a part of the media or intima, were observed. The region of the brain stem and spinal cord appeared more liable to be affected than other parts of the brain and corresponded with the greatest deposition of pus.

III. *Late stage*

Case 5. Frontal sinusitis. Purulent lepto- and pachymeningitis (pneumococcus, type 7)

A. H., aged 22, a previously healthy young soldier, developed acute right frontal sinusitis. He was treated with sulphapyridine (34 g.), and after 12 days he seemed to be recovering when he developed purulent lepto- and pachy-meningitis (pneumococcus, type 7), which began with headache, fever, a generalised fit, paralysis of the left leg and weakness of the left arm. He was treated for 4 days with sulphathiazole (29 g.), and the right frontal sinus was explored and an extradural abscess evacuated. The leptomeningitis was intense, producing paralysis of oculomotor nerves and signs of severe damage to the anterior horn cells and spinal nerve roots. On the fifth day of the meningeal infection he was delirious, restless and screaming, with intense neck rigidity, fixed dilated pupils, motionless eyeballs, flaccid left hemiparesis and hemianalgesia, and severe abdominal distension; spinal fluid flowed freely and contained ¾ volume solid pus. Blood culture negative. Penicillin was given by lumbar puncture for 7 days (total 34,500 units) until the flow of C.S.F. stopped, presumably from adhesions between arachnoid and pia. Systemic penicillin was given for 21 days (total 2,770,000 units).

After 2 days of intrathecal and 1 day of parenteral treatment, the C.S.F. became sterile. However, signs of involvement of the spinal cord and roots extended: the left leg became completely paralysed, most of the tendon jerks

in the lower limbs were lost and there was retention of urine, while the right side of the diaphragm also became paralysed.

On the 10th day of meningitis, in order to inject penicillin into the ventricles, parietal burr holes were made and it was found that he had free pneumococcal pus (8 c.c. +) in the subdural space on the left side; this was successfully treated by local instillations of penicillin solution through an indwelling catheter (45,000 units in 8 days). No pus was found in the subdural space on the right side. Five thousand units of penicillin were injected into the lateral ventricle; this route was not again used, because the right ventricle was always difficult to find and the left was not tapped because of subdural pus over the left hemisphere.

By the 12th day of meningitis, when lumbar puncture was yielding dry taps, the patient was considerably improved: he smiled and spoke, his sight was impaired but was improving, his pupils were beginning to react to light, oculomotor palsies were still gross but were beginning to recover; his limbs and trunk presented a picture resembling recovering poliomyelitis—profound muscular wasting, with absent tendon jerks, poor peripheral circulation and also retention of urine. Blood urea 80 mg. per 100 c.c. on 15th day. He became sleepless and mildly poikilothermic, and his tongue was raw and red. On the 27th day he suddenly developed a mild diabetes insipidus (output 150 oz. in 24 hours).

Partial recovery of all muscular paralyses continued, and his temperature had been practically normal for two weeks, with pulse steady though on a high level when, on the 35th day of the meningeal infection, he developed signs of a right-sided brain abscess. One hundred c.c. of subdural pus on the right side of the falx cerebri were aspirated through the medial part of the right parietal lobe; the pus showed Gram-positive cocci in films but no growth in cultures. He improved, but relapsed. A further attempt was made to drain the abscess without success, and he died 7 weeks after the onset of meningitis. A high blood pressure ranging up to 225/158 mm. was a feature of the final phase of the illness, though after withdrawal of the pus it fell to 136/80 for a short time.

Necropsy. (P.M. 234. 1943). *Septicæmia. Subdural abscess. Purulent leptomeningitis. Left frontal sinusitis.*

Obsolete tuberculous focus in lower lobe of left lung. Moderate œdema and congestion of lungs. Dilatation of right ventricle of heart. Septic spleen. Chronic purulent cystitis. Ascending purulent pyelonephritis with moderate dilatation of pelvis and ureter of both kidneys. No further abnormalities in abdominal or thoracic organs.

There was chronic osteomyelitis of the posterior wall of the right frontal sinus, which contained a small amount of grey gelatinous material. A subdural abscess (up to $3 \times 2 \cdot 5$ cm. in the coronal plane) extended along the right border of the superior longitudinal sinus to gain the medial and ventral surfaces of the right occipital lobe and ended in the right middle fossa over the hippocampus and inferior temporal convolution (fig. 7). Dense fibrous adhesions united brain, falx and tentorium throughout this area and the right lobe of the cerebellum was similarly adherent to the tentorium. The wall of the abscess was composed of dense grey fibrous tissue (up to 0·25 cm. thick on the dural aspect and about 0·1 cm. thick on the arachnoid aspect) and the contents consisted of thick grey pus. The adjacent

cerebral convolutions were considerably deformed by pressure. Gummy adhesions united the arachnoid membrane and clivus.

Apart from puncture wounds, no further visible abnormality was found in the substance of the brain, including the ventricular system. Dense grey fibrous adhesions linked the dura and arachnoid membranes throughout the posterior aspect of the spinal cord. A small pocket of pus occupied the lumbar subdural space but no further pus was found in the theca. The roots of the cauda equina were densely matted by fibrous tissue apart from occasional spaces containing clear fluid. There was less conspicuous fibrosis of the subarachnoid space at higher levels of the cord, the posterior aspect being mainly involved. Numerous disseminated pin-point and pin-head orange flecks occupied the white matter beneath the pia in the cervical and upper thoracic segments, becoming fewer in the lower thoracic and absent from the lumbar and sacral segments. There was considerable diffuse softening of the substance of the cord.

Microscopical examination. Sections of the kidney show a focal purulent ascending pyelonephritis. There is no visible alteration of the blood vessels in the kidney, pancreas and suprarenal. The splenic pulp is acutely inflamed.

The dura over the left cerebrum is slightly thickened by the formation of young granulation tissue, sparsely infiltrated with lymphocytes and plasma cells, over its inner surface. There are no vascular changes. The subdural abscess on the right side is enclosed by a dense wall of fibrous granulation tissue, and there is great fibrous thickening of the adjacent leptomeninges. The underlying convolutions are compressed and show severe diffuse neuronal degeneration accompanied by foci of softening. Beneath the pia there is proliferation of the perforating vessels and of the microglia. In some of the leptomeningeal arteries of this region the endothelium is raised over an eosinophilic coagulum, and in a few the intima is slightly thickened by spindle cells.

There is advanced chromatolysis in the tuberal nuclei of the hypothalamus, and less marked of the paraventricular and supraoptic nuclei. The leptomeninges here are sparsely infiltrated with lymphocytes, large mononuclear cells and (fewer) neutrophil leucocytes. The infiltration extends into the tuber to reach the floor of the third ventricle. A few vessels at the upper end of the pituitary stalk have undergone recent thrombosis accompanied by hæmorrhage into the intima and early fibrinoid necrosis of the media. The site of the needle track entering the right frontal lobe is occupied by degenerating leucocytes; much of the ependyma here is missing. The choroid plexus from a lateral ventricle is œdematous, but its stroma is otherwise normal. The epithelium shows severe focal degeneration with pyknosis and karyolysis; in places the epithelium is missing; elsewhere the cells are greatly flattened in a manner suggesting regeneration, but no mitoses are found. A little cellular debris

containing an occasional neutrophil leucocyte and a few Gram-positive cocci in pairs and short chains are present between the villi.

Over the base of the pons there is considerable thickening of the leptomeninges by young collagenous tissue unevenly infiltrated with lymphocytes, a few plasma cells and large mononuclear cells. Many perforating vessels have undergone fibrinoid necrosis and thrombosis, associated with perivascular zones of softening and hæmorrhage which are infiltrated with cells of microglial character (fig. 8). In the meningeal vessels, notably the basilar artery, there is focal cellular hyperplasia of the intima without necrosis or cellular infiltration.

Throughout the cord there is collagenous thickening of the pia and a rather sparse uneven infiltration of the pia-arachnoid with small lymphocytes and large mononuclear cells, almost confined to the posterior surface. A few extra- and intracellular Gram-positive cocci are present. In an upper thoracic segment the small arterioles of the pia, and particularly in the adjacent nerve roots, show fibrinoid necrosis of part or the whole of the circumference of the media, accompanied by swelling, great vacuolation and cellular hyperplasia of the intima. Some have the intimal change alone. Affected parts of the nerve roots are infiltrated with round cells and the fibres are in various stages of degeneration. In the white matter of the cord, especially of the posterior and lateral columns, there are about 15 ill-defined wedge-shaped patches of softening of variable size infiltrated with microglial cells. A few smaller foci lie more deeply in the lateral columns. Both posterior horns are necrotic, and there is severe chromatolysis of the anterior horn cells. No definite changes are present in the perforating vessels.

In a lower thoracic segment, with the attached dura, the vascular changes are more conspicuous and affect both meninges and cord. The dura is thickened by fibrosis and an uneven, mainly perivascular, granulomatous cellular infiltration associated with recent diffuse hæmorrhages: Almost all the dural vessels show conspicuous hyperplastic thickening of the intima, sometimes almost obliterating the lumen. In some a dense zone of fibrin occupies the innermost part of the intima (*cf.* fig. 9); in others a crescentic mass of fibrin lies in the depths of the thickened intima. In a few there is recent hæmorrhage just beneath the endothelium (fig. 10). Similar vascular changes are present in the pia, in the nerve roots and in the cord, where affected vessels are surrounded by aggregates of microglial cells (fig. 11). The smaller arterioles, as shown in this photograph, usually display fibrinoid necrosis of the whole thickness of the wall. In the substance of the cord there is œdematous softening and severe chromatolysis of the neurones, with a few small ill-defined areas of necrosis.

In the lumbar region the vascular changes in the dura are similar; but in the leptomeninges and nerve roots they are less conspicuous; in the cord they are restricted to necrosis of a few perforating vessels

PLATE CXXV

ARTHRITIS IN PNEUMOCOCCAL MENINGITIS

FIG. 8.

FIG. 9.

FIG. 10
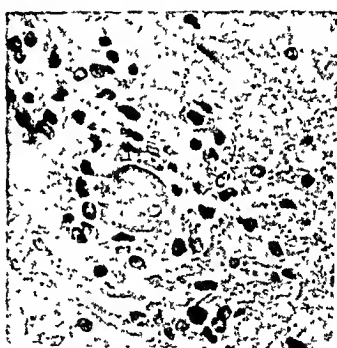
FIG. 11
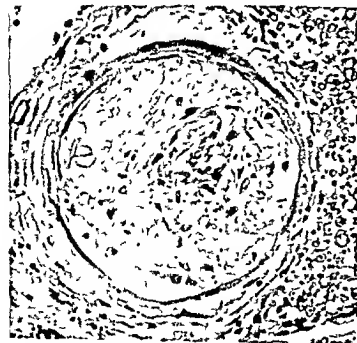
FIG. 12.

FIG. 13.

in the border of one anterior horn. The anterior horn cells are well preserved except for a few which show chromatolysis.

In the cauda equina the roots and meninges are matted by very vascular fibrous granulation tissue which is unevenly infiltrated with lymphocytes and large mononuclear cells. The dura is densely infiltrated in places with similar cells. Obliterative and necrotising vascular changes of the kinds already described are conspicuous in all areas. In the nerve roots advanced endarteritis (fig. 12) is accompanied by degeneration of the fibres in many areas (fig. 13).

In this case the infection of the meninges was intense and led to purulent pachymeningitis as well as leptomeningitis. The subdural infection over the left cerebral hemisphere subsided completely after evacuation of the pus by operation and instillation of penicillin into the subdural space through an in-dwelling catheter. The subdural infection on the right side was not discovered until a few days before death, by which time there was a large subdural abscess on the medial and inferior aspects of the right cerebral hemisphere.

Owing to shortage of supplies systemic penicillin was stopped on the 24th day of the meningitis and it was 11 days after this that the right subdural abscess produced symptoms of raised intracranial pressure which led to death. During this final phase the blood pressure was unusually high. This is not usually seen in raised intracranial pressure of gradual onset and is probably attributable in this case to the severe changes in the arteries of the central nervous system.

Signs of the severe degenerative and necrotic changes in the nerve roots and grey matter of the spinal cord, with a clinical picture resembling poliomyelitis, were present within 2 weeks of the onset of meningitis, thus suggesting that the vascular changes in the spinal meninges and nerve roots were already present at an early stage of the illness. The poikilothermic tendency and the diabetes insipidus which were observed in the fourth week of the meningitis were in accord with the changes in the hypothalamus and pituitary stalk. To what extent these changes were due to necrotising arteriolitis is uncertain, since those observed histologically appeared to be of recent origin.

Case 6. Pneumococcal meningitis (pneumococcus type 13).

J. W. (R.I. 17936/43), male aged 15, four years before had had a left subdural abscess, post-operative brain fungus and leptomeningitis (*H. influenzæ*) as complications of mastoiditis. The pneumococcal meningitis followed recurrence of middle-ear infection and lasted 53 days from onset to death. On the first day of the disease the patient received 6 g. of sulphapyridine, from the 2nd to the 7th day 60 g. of sulphadiazine, and thereafter until death 71,000 units of penicillin intrathecally by various routes. During the meningitis the area of the old healed brain fungus bulged, the thin scar on its surface became necrotic and ulcerated, and eventually a pneumococcal abscess formed in the underlying brain, at the site of what had been shown by air studies 4 years previously to be a brain cyst.

Necropsy (*P.M.* 536. 1943). Fibrous pleural adhesions over apices of upper lobes of both lungs. Basal bronchopneumonia and hypostatic congestion of left lung; acid digestion of lower lobe of right lung. No further abnormalities in other thoracic and abdominal organs. An emaciated young man with greater wasting of right leg than of left.

A soft, fluctuant, elliptical area of greyish-pink wrinkled epithelium, 13×4 cm. and corresponding to the site of the fungus cerebri, occupied the scalp behind the left ear over a defect, 10×4 cm., in the bone, including the mastoid antrum. The centre of the epithelium was superficially ulcerated. The cerebral convexities were dry and united in many places to the dura by delicate fibrous strands. Over the brain stem these were denser, and the leptomeninges here were matted by grey fibrous tissue containing pockets of thick pus obscuring the nerve roots. Similar but non-purulent tissue occupied the basal cistern and proximal ends of the Sylvian fissures. The floor of the third ventricle was ballooned and there was great softening and fluctuation of the left parieto-occipital region immediately dorsal to the fungus. The arachnoid membrane over the cisterna magna was grey and opaque, the cistern itself being filled with thick pus.

On section a cavity, $2 \cdot 8 \times 2 \cdot 2 \times 3 \cdot 8$ cm., containing pultaceous exudate occupied the left cerebrum beneath the fungus and communicated with the vestibule of the lateral ventricle by an orifice $0 \cdot 5$ cm. in diameter. The cavity was lined with tough pinkish-grey tissue of wash-leather consistency up to 1 cm. thick. Gross hæmorrhagic softening affected the adjacent white matter. Thick pus filled the occipital horns and formed a membrane over the ependyma, plugging the right foramen of Monro and occluding the aqueduct (fig. 14) and fourth ventricle. The whole of the ventricular system was dilated, especially the left lateral ventricle.

Numerous petechial hæmorrhages mottled the subependymal white matter and, over the right temporal horn, were accompanied by gross softening. The lining of the fourth ventricle was replaced by a soft pinkish-grey membrane about $0 \cdot 05$ cm. thick, and all the foramina were sealed by gelatinous grey tissue which, in the left foramen of Luschka, contained a thrombosed vessel with thick grey walls. A few recent hæmorrhages, up to $1 \times 0 \cdot 5$ cm., occupied the medulla oblongata beneath the hind end of the ventricle. The basilar artery was patent.

Tough, fibrous adhesions united the dura and arachnoid over the upper cervical segments of the cord and throughout the terminal 8 cm. of the cauda equina. Some recent clot occupied the subdural space over the upper cauda equina. A thin film of grey exudate covered the whole length of the cord in the subarachnoid space, being most marked in the thoracic segments. The substance of the cord was uniformly rather soft and the demarcation between grey and white matter indefinite, especially in the cervical segments.

Microscopical examination. The surface of the fungus is epi-

ARTERITIS IN PNEUMOCOCCAL MENINGITIS
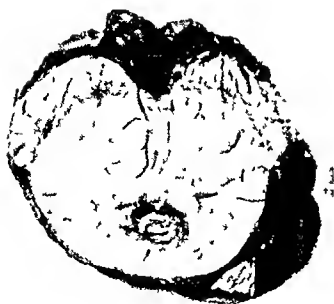
Fig 14 —Case 6   Obliteration by inspissated
pus of dilated aqueduct of Sylvius

Fig. 15 —Case 6   Endarteritis in chronically
inflamed meninges over medulla oblongata
H and E   ×105

Fig 16 —Case 6   As in fig 15, showing
endarteritis, and, in central vessel, obliteration
of lumen by old organised thrombus.
Weigert's fuchselin and neutral red   ×100
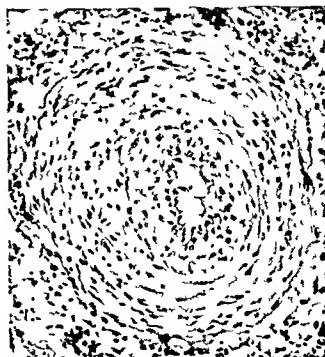
Fig 17 —Case 6   Endarteritis in sheath of a
nerve root in the cauda equina. H and E
×115

thelialised and beneath this the brain tissue is completely destroyed by fibrous granulation tissue to within about 1 cm. of the lateral ventricle. There is necrosis and purulent infiltration of this juxta-ventricular tissue but no organisms are demonstrated. Obliterating endarteritis fibrosa is present in a group of vessels near the deep border of the granulation tissue. The leptomeninges over the frontal region appear normal, but towards the base—for example over the right temporal lobe—there is slight infiltration with large mononuclear cells without fibrosis. Over the mid-brain fibrosis is slight, areas of old pus lie beneath the arachnoid membrane and the pia is infiltrated with lymphocytes and large mononuclear cells, some of which have ingested red corpuscles. About the fourth ventricle fibrosis is great. The tissue is infiltrated with large mononuclear cells, neutrophil leucocytes, plasma cells and lymphocytes. In the meninges vascular changes are limited to the region of the fourth ventricle. Here endarteritis fibrosa (figs. 15 and 16) is conspicuous and in some instances there is evidence of thrombosis with recanalisation. Earlier stages of endarteritis are also present. In other arteries, of all calibres, there is hypertrophy of the intima with reduplication of the elastica; it is focal in the basilar artery. The dorsal half of the medulla oblongata, the walls of the fourth ventricle, flocculi and central white matter of the cerebellum contain areas of recent haemorrhagic and anaemic softening, some of which are infiltrated with macrophages. Haemorrhages are present in the mid-line near the median raphe' in association with thrombosis of a few of the perforating veins.

The ependyma of the fourth ventricle and aqueduct is occupied in many places by macrophages. These cells abound in the sub-ependymal glia and some have gained access to the adjacent pus in the lumen. The subependymal vessels are cuffed with lymphocytes. Similar changes are present in the lateral ventricles, where much of the ependyma has been destroyed. The choroid plexus of the fourth ventricle shows focal purulent infiltration of the stroma and hyaline-droplet degeneration of the epithelium. The plexus of the left lateral ventricle is matted with pus, fibrin and young granulation tissue, but the villi show little change beyond focal leucocytic infiltration of the stroma. Feebly staining Gram-positive cocci are present in small numbers in the meninges of the mid-brain and in the pus within the ventricles.

Meningitis similar to that seen over the medulla oblongata is present throughout the length of the cord. In the cervical region and lower cauda equina the dura and pia-arachnoid are fused by fibrous granulation tissue, while in the thoracic and lumbar segments there is less fibrosis and the subarachnoid space contains foci of old pus. Apart from a little round-cell infiltration of its inner layers, the dura where adherent is unaltered. The nerve roots are not obviously affected. The cervical and upper thoracic segments show severe

central softening of the cord, involving all the grey matter exce[
the lateral fringes of the anterior horns. In the softened area the[
are slight diffuse recent hæmorrhage, sparse infiltration with lar[
mononuclear cells and a few groups of neutrophil leucocytes. T[
white matter at the periphery of the cord shows well defined wedg[
of *ischæmic degeneration* in which the axis cylinders are great[
swollen or destroyed and the myelin sheaths conspicuously balloone[
In the lower thoracic and lumbar segments the cord appears norm[
apart from chromatolysis of the neurones.

At all levels some of the pial arteries show variable degrees [
endarteritis fibrosa and, in one artery in a cervical segment, this [
associated with mucinous degeneration of the deeper parts of th[
thickened intima. Occasionally, especially in the small arteriole[
there is great stenosis. No fibrinoid necrosis or thrombosis [
observed in these arteries, and no changes are found in the perforatin[
vessels. In general the changes tend to diminish towards the caud[
segments, but a single artery in the lower cauda equina shows marke[
endarteritis (fig. 17). There is also thrombophlebitis, with organisatio[
and recanalisation, of a large epidural vein at this level.

Comment on late stage

In these two cases the meningitis was protracted for 7-8 weeks.
The principal histological difference between this and the intermediate
stage lies in the conspicuous evolution of proliferative vascular
changes in this late stage ; these are particularly well displayed in
case 5. In both these cases endarteritis fibrosa is pronounced, but
progression is shown by the presence also of fibrinoid necrosis, either
of the intima or of the whole wall in the small arterioles (figs. 8 and
11), as well as by the escape of red corpuscles into the intima (fig. 10).
These vascular changes are promiscuously distributed, but predominate
in the brain stem and spinal cord, a tendency that was already in
evidence in the intermediate stage. In association with these, and
obviously referable to them, there was widespread softening of the
spinal cord in both cases and, in case 6, medullary hæmorrhages to
which death was probably due.

DISCUSSION

The cases described have been divided into three groups according
to the stage reached by the meningitis. In the early stage, with
death within a few days of the onset, vascular changes were principally
found in the leptomeninges over the cerebral convexities, where the
pus was most abundant. Here thrombophlebitis and early purulent
endarteritis affected the larger vessels. Fibrinoid necrosis of small
arterioles was present beneath the ependyma in association with
necrotising ependymitis. That these changes were due to the

infection is proved by their identification in similar early cases untreated by any form of chemotherapy. It is instructive to observe how cases of pneumococcal meningitis in the pre-chemotherapeutic era invariably came to necropsy within ten days of the onset of the disease, and mostly within a much shorter time.

The more prolonged infections, as seen in the intermediate and late stages, represent a form of reaction rendered possible by chemotherapy. In the intermediate stage the duration of the infection was about two weeks. The meningeal inflammation had by then become subacute. But the vascular changes were similar in general character to those observed in the early stage. The tendency for fibrinous deposits to appear in the walls of meningeal arteries, with or without accompanying foci of purulent panarteritis, and the predominance of such lesions in the region of the brain stem and spinal cord rather than over the cerebral convexities, are of interest when these cases are compared with those in the late group, and indicate both the tendency for vascular lesions to develop where pus stagnates and also the continuity between our various stages. On the other hand it must be admitted that the vascular changes of the intermediate stage were by no means conspicuous; also that the two cases mentioned form a somewhat slender link between the early and late stages. They are, however, closely similar in one late case, not described in this paper, in which the patient survived for six weeks after the onset of the infection. In this case it is noteworthy that the vascular changes were confined to the spinal cord, and were again associated with softening and haemorrhage in the cord, which had obviously resulted from circulatory disturbance.

The changes we have described in the early stage have been cursorily noted by others in association with purulent meningitis (Untersteiner, 1926; Neal, Jackson and Appelbaum, 1931; Winkelman and Eckel, 1935), and endarteritis fibrosa has been found in the chronic healing phases of some forms of purulent meningitis, especially those due to the meningococcus (Kaufmann, 1911) and in streptococcal meningitis (Turnbull, 1914-15), but the degrees seem to have been slight. So far as we are aware the gross proliferative and necrotising changes which we have observed in our late cases have not previously been reported in association with purulent meningitis.

These late cases (nos. 5 and 6) show progressive vascular changes which include fibrinoid necrosis, either alone or in combination with endarteritis fibrosa, and endarteritis fibrosa with or without thrombosis. There is perivascular inflammatory-cell infiltration. Collectively the picture closely resembles that seen in many cases of polyarteritis nodosa. Haemorrhage and softening were found in many parts of the brain stem and spinal cord in association with these vascular changes, which were thus in large measure responsible for death.

The resemblance between these late vascular changes and those found in polyarteritis nodosa and in the granulomatous forms of

meningitis, especially tuberculosis, suggests a similar mode of production. Rich and Gregory (1943) have demonstrated the role played by tissue sensitisation in the ætiology of polyarteritis nodosa, and it is possible that repeated reinfection of the cerebrospinal pathway from an unresolved focus may similarly have been responsible in our late cases. On the other hand we have seen several patients who have recovered completely from more than six successive attacks of pneumococcal meningitis (Smith *et al.*) without any clinical residuum suggesting arterial damage. And in case 5 the clinical findings suggest that degeneration of the spinal grey matter and nerve roots began during the initial attack of meningitis, though it is possible that inflammatory infiltration of the nerve roots was the first change. In case 6 there was an attack of meningitis 4 years before death : this, however, was due to *H. influenzæ* and, while some part of the chronic inflammatory changes in the meninges may be referable to this attack, it does not help in explaining the more recent vascular developments.

Again, the sulphonamides have been incriminated in the development of these vascular lesions as a result of sensitisation. But in cases 5 and 6 this form of chemotherapy was discontinued when penicillin was begun, since at that time we were testing the therapeutic value of penicillin. Thus the question of sensitisation by sulphonamides does not arise.

The clinical significance of these findings cannot as yet be determined. With few exceptions recovery in the survivors of pneumococcal meningitis in our series treated with penicillin has been complete (Smith *et al.*). In one case, however, the evidence suggests that decerebrate rigidity with posterior basic meningitis, hitherto supposed to be due to hydrocephalus, may be the result of a circulatory disturbance in the brain stem ; for this patient, who had a blocked subarachnoid space when penicillin treatment was begun 4 weeks after the onset of meningitis, continued to show severe extensor rigidity of the limbs months after the circulation of the cerebrospinal fluid had been restored to normal. This case, like the fatal cases described in the present paper, was an example of an intense infection.

SUMMARY

Penicillin therapy, in a few unsuccessfully treated cases of pneumococcal meningitis, has permitted the observation of changes occurring in the late stages of infection.

In those which have progressed for 7 or 8 weeks, conspicuous and progressive vascular changes resembling those of polyarteritis nodosa have been identified in the central nervous system. They have been accompanied by hæmorrhage and softening of the adjacent nervous tissues.

Study of the earlier stages of pneumococcal meningitis suggests

that these late vascular changes are probably evolved from the earlier, but tissue sensitisation through repeated reinfection of the cerebro spinal pathway from an unresolved focus is a possible factor in their production

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BASOPHILISM AND CARCINOMA OF THE PANCREAS

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CUSHING considered that the syndrome which he called basophilism was caused by an adenoma of the basophil cells of the anterior lobe of the pituitary gland. A similar clinical picture had already been described in two patients by Leyton, Turnbull and Bratton (1931) in association with carcinoma of the thymus and subsequently others were reported with tumours of the adrenal cortex or the ovary, or with no tumour in any of these organs. In 1935 the author described. a hyaline change in the basophil cells of the anterior lobe of the pituitary gland which is now accepted as the only common pathological change common to all forms of basophilism, but the part which these cells play in the causation of a disease with such varied pathological changes is still controversial. In this paper, therefore, a case of basophilism associated with a tumour of the pancreas is reported in the hope that, by adding more material to the already heterogeneous collection, we may arrive at a clearer conception of the altered physiological activity in this condition.

Case report

Clinical history

W. D., a single woman aged 28, was admitted to the London Hospital on 23rd June 1945. She gave no relevant family history and had had no serious illness until six months previously, when she had begun to have vague abdominal discomfort unrelated to meals but associated with occasional vomiting. At this time she noticed that her face was becoming darker and she began to put on weight. Two months before admission she stepped on a nail which she extracted from her foot by herself. She was given anti-tetanic serum and subsequently developed a rash on her arms, chest and abdomen which eventually cleared up completely, but she was also having a bromide tonic at this time. She was sent home and her mother noticed that her face had become swollen, darker and more hairy, although she had noticed a little hair on her lips for the previous two years. Her periods, which had been regular, ceased abruptly two months before admission. She also remarked that her legs were getting thinner and a week before admission she complained of aching in the legs and tightness in the chest, with some difficulty in breathing. She was also becoming thirsty, had some frequency of micturition and was very constipated, and she said her sight was deteriorating.

On examination she seemed rather dull and apathetic. She had a full face with a dusky, plum-coloured, somewhat greasy skin. She had a moderate amount of dark hair on the upper lip and chin and male distribution of hair on the abdomen. There was a small red macular rash scattered over the front of the upper part of the thorax. There were no striæ or bruising and the clitoris was not enlarged. The tongue was furred and the breath foul. There was no enlargement of the lymphatic glands. The systolic blood pressure varied from 145 to 190 mm. Hg., the diastolic from 80 to 100. The heart and lungs were otherwise normal. In the abdomen the liver edge was palpable and there was a diffuse ill-defined mass palpable just below it. At first this mass was considered to be an adrenal cortical tumour but the urinary output of 17-ketosteroids, which was 15 mg./24 hours, made this diagnosis unlikely. The urine contained a cloud of albumin on admission and glycosuria was present continuously. The blood sugar was 100 mg./100 c.c. fasting, but it rose progressively to 250 mg./100 c.c. two hours after the oral administration of 50 g. of glucose. The hæmoglobin was 110 per cent. and the total white cell and differential counts were normal. The blood urea was 30 mg., plasma chlorides 561 mg. and serum calcium 10·3 mg./100 c.c. An X-ray of the sella turcica was normal. Four days after admission she developed jaundice which increased rapidly. Eleven days after admission she began vomiting foul black material and her urinary output fell from an average of 86 oz. daily to 42 oz. on the tenth day, 2 oz. on the eleventh and none the twelfth day. She died at 12.10 a.m. on the thirteenth day after admission.

Summary of necropsy (*P.M.* 136, 1945)

Obstructive jaundice. Carcinoma of head of pancreas associated with basophilism. Scirrhous carcinoma, measuring 4·5 × 4 cm., replacing greater part of head of pancreas and constricting, through invasion, lower end of common bile-duct. Great dilatation, 4 cm. in circumference, of common bile-duct above obstruction, and of cystic and right and left hepatic ducts. Hæmorrhagic secondary growth in serosa and wall of gall-bladder just below neck, with perforation of mucosa and dilatation of gall-bladder by fluid blood and clot mixed with a little bile. About 3 oz. of blood clot in peritoneal cavity. Numerous nodules of secondary growth throughout liver, most of them firm and white, a few hæmorrhagic and cystic. A few small nodules of secondary growth in peritoneum of pouch of Douglas. Deep jaundice of liver tissue. Jaundice and severe parenchymatous degeneration of kidneys. Moderate jaundice of skin and marked of conjunctivæ. Dilatation of stomach and ileus paralyticus of small intestine. Very severe acid digestion of lungs. Normal spleen. Diffuse hyperplasia of cortex throughout both suprarenal bodies, with diminution of lipoid and poorly developed pigment zone. Abundant colloid in normal thyroid. A little glandular tissue in mainly adipose thymus. No visible macroscopic abnormality in pituitary or parathyroids. Slight active and chronic rheumatic endocarditis of tricuspid valve and chronic of mitral. Slight hypertrophy without dilatation of left ventricle. Slight general atheroma. No abnormality in pelvic organs or external genitalia. No evidence of osteoporosis in right femur, tibia, humerus, ribs or vertebræ. No

abnormality in brain. Considerable obesity of whole body. Plump face with pronounced moustache (1 cm. long) and less marked hirsuties over rest of face. Hair all over lower abdomen and pubes. Acneiform eruption in skin of back. A well developed, muscular woman.

Weights. Body 135 lb. 12 oz., length 5 ft. 5 in., heart 11¾ oz., liver 3 lb. 15¾ oz., kidneys 12¼ oz., suprarenals—left 11·8 g., right 13·1 g., spleen 2¾ oz., brain 2 lb. 12½ oz., pituitary 0·68 g., thymus 13·9 g., thyroid 28·1 g., ovaries 8·4 g.

Microscopic examination

Pituitary. The gland was divided horizontally into two equal parts after formol fixation and these were embedded together. Serial sections were made and stained by a modification of Mallory's acid-fuchsin anilin-blue (Crooke and Russell, 1935). There is no obvious disparity in the numerical proportions of the different types of cells in the anterior lobe. The acidophil and chromophobe cells appear normal but, with rare exceptions, all the basophil cells show grades of the hyaline change characteristic of Cushing's syndrome (Crooke). The change is conspicuous in most. Occasional basophil cells are of giant size and contain 3 or 4 nuclei. No adenoma is seen, but four foci of hyperplasia of chromophobe cells are present in the anterior lobe. The largest, measuring 1·6 × 1·4 mm., is composed of small, greatly vacuolated cells and fewer elongated columnar cells containing pale-grey cytoplasmic granules. The smaller foci are all composed of these columnar cells. All foci contain sparse unevenly distributed acidophil and basophil cells. There is no abnormality in the posterior lobe.

Pancreas. The tumour is composed of abundant dense collagenous tissue, embedded in which are trabeculæ and rounded areas of well defined small polygonal cells with finely vacuolated, lightly eosinophil cytoplasm. The nuclei have lightly staining nucleoplasm, traversed by a delicate net of chromatin beset with nodes of various sizes, and they contain a small nucleolus. Much pyknosis is present. The cells often form duct-like tubules, the lumen being either empty or partly filled with a little eosinophil debris. The tumour infiltrates the adjacent pancreatic tissue, which in other respects appears normal. With Masson's trichrome stain the islets are well demonstrated, and a considerable number of these persist unchanged in an area of the tumour remote from the more normal pancreas. Whereas the granules of the islet cells are specifically stained either by acid-fuchsin or anilin-blue in this technique, the tumour cells proper display a purplish-grey cytoplasm and resemble, in this respect, the cells of the pancreatic acini. With Vines's ponceau-fuchsin method the staining of the islet cells is not so distinctive ; occasional cells of the pancreatic acini contain brightly fuchsinophil zymogen granules,

but no granules are demonstrated in the tumour cells. There is no alteration in the blood vessels of the non-infiltrated pancreas.

Sections from the *liver* and a *cœliac gland* show secondary carcinomatous deposits of similar histological character to the primary growth.

Suprarenals. The hyperplasia of the cortex mainly affects the zona fasciculata which is greatly deepened. There is great engorgement of the zona reticularis. In frozen sections stained with Sudan III the amount of lipoid is small; it is mainly confined to small groups of cells in the outer third of the zona fasciculata but is present also in occasional cells of the zona reticularis. The capsular arterioles appear normal.

Kidneys. There is severe albuminous and dropsical degeneration of the epithelium of the convoluted tubules and loops of Henle and of Bowman's capsule. Islands of first convoluted tubules have frequently undergone necrosis. There is slight diffuse fatty degeneration in the loops of Henle and second convoluted tubules. The latter often contain bulky hyaline and granular bile-stained casts. The collecting tubules in the medulla contain more numerous, similar casts. There is no further abnormality in the glomeruli, interstitial tissue or blood vessels. In particular there is no histological evidence of persistent high blood pressure.

No abnormality is present in the *thymus, thyroid* or *parathyroid glands* (left lower parathyroid not identified). The *ovaries* contain primary and atretic follicles together with a few small follicular cysts. No corpus luteum is seen.

DISCUSSION

The association of basophilism with carcinoma of the pancreas is interesting because similar cases have been reported previously. In 1933 Kepler described the first example in a woman aged 30 years, who had a fat florid face but had lost a considerable amount of weight. She had hirsuties of the upper lip and chin, hyperglycæmia, glycosuria and amenorrhœa and she gave a positive Friedman test, but no mention was made of pregnancy. An adrenal tumour which was removed surgically was apparently a metastasis from a carcinoma of the head of the pancreas which was found at autopsy, but full histological details are not available. McLetchie and Scott (1942-44) described a woman aged 26 years with basophilism associated with a tumour in the pancreas which they believed to be derived from an adrenal cortical rest because its cells contained granules which took an acidophil stain by Vines's method. It is noteworthy, however, that the zymogen granules of normal pancreatic cells also take this stain and it is possible that this tumour was a primary pancreatic carcinoma. She had a typical dusky appearance, hirsuties, acne vulgaris, amenorrhœa and slightly raised blood pressure. In 1945 Mellgren described another example in a woman aged 50 years with

a "moon face" and typical obesity of the "buffalo" type, hypertension and hyperglycæmia. The menopause occurred three years previously. The characteristic hyaline basophil cells were found in the pituitary glands of the cases described by McLetchie and Scott, and by Mellgren; unfortunately the gland was not examined in Kepler's case. There was no detailed description of the pancreatic tumour in either Kepler's or Mellgren's case.

The most striking feature about our patient and those reported in the literature is that three of the four were aged 30 years or younger, an age at which carcinoma of the pancreas is very rare. We confirmed this by analysing the post-mortem reports of carcinoma of the pancreas at the Bernhard Baron Institute of Pathology at the London Hospital. There have been 102 cases reported between 1907 and the present time and the youngest female was aged 35, the youngest male 32 years. All four patients with basophilism associated with carcinoma of the pancreas were females. We now attempted to examine the question statistically and found from the reports of the Registrar General (1938) that in the three years 1930-1932 15 women aged 25-35 years were certified in England and Wales as dying from carcinoma of the pancreas. In the 1931 census there were 3,308,000 women (neglecting the hundreds) between the ages of 25 and 35 years. The chance of a woman being registered as dying from carcinoma of the pancreas between the ages of 25 and 35 years is thus roughly $\frac{10}{3} \times \frac{15}{3,308,000}$ or 1 in 66,000. The number of patients with basophilism is difficult to assess. Thompson and Eisenhardt (1943) in their review found a total of 98 autopsies reported in the world literature up to 1940. The average length of survival after the development of basophilism is probably under five years. If we assume therefore that 1000 cases of basophilism have survived for ten years from the age of 25-35 years, the chance of the group providing a single death from carcinoma of the pancreas is 1 : 66 or 0·015. The chance of two deaths from carcinoma of the pancreas is (0·015)² or 0·000225 and the chance of three deaths is 0·000,003,375. Even if we assume that 10,000 cases of basophilism have been watched from the ages of 25-35 years, the chance of two deaths from carcinoma of the pancreas is still only 0·0225, and of three deaths 0·003,375.

It might be objected that the registrations of death from carcinoma of the pancreas in the age group 25-35 are far below the truth but this is unlikely, because the incidence of carcinoma of the pancreas in the younger age groups is approximately constant for parts of the world where statistics are available; it is constant for the different social classes; it has remained the same for the last 30 years and it conforms with the findings in our carefully examined series. If the certifications were grossly incorrect the incidence would vary with the frequency of post-mortems at different times and in different places. On the other hand the incidence of carcinoma of the pancreas

rises so steeply with age that the probability of death from this disease between the ages of 25 and 30 years would appear to be only about one-sixth of the probability between 25 and 35 years. So that even if the true incidence is six times higher than the Registrar General's figures the validity of our calculation is not affected.

Since it has been shown statistically that the association of basophilism with carcinoma of the pancreas is not fortuitous it is pertinent to seek for a cause. Now basophilism may occur without a tumour in any organ, but in a series of 98 cases reported in the literature Thompson and Eisenhardt found only twelve with no tumour anywhere. In 60 cases there was a tumour in the pituitary gland, usually composed of basophil but sometimes of chromophobe cells, in 22 there was a tumour of the adrenal cortex, in three of the thymus, and in one of the ovary. All the pituitary tumours except two were benign, six of the 22 adrenal cortical tumours were benign and the other 16 together with the three thymic and one ovarian were malignant. Thus there was no tumour in 12·3 per cent., a benign tumour in 65·3 per cent. and a malignant tumour in 22·4 per cent. of the cases. All these tumours were in endocrine glands, but there is no evidence to suggest that any of the four pancreatic tumours associated with basophilism were related to the endocrine components of the pancreas. One must conclude that basophilism is a disorder usually associated with tumour formation but the mechanism remains obscure. Theoretically it may be suggested either that the pituitary gland is producing a growth-stimulating substance causing tumour formation directly, or that its trophic hormones are stimulating the production of steroids in other glands, and that these or their breakdown products may be responsible for tumour formation, since it is well known that some of the steroids are carcinogenic in certain strains of mice.

SUMMARY

A case of basophilism associated with carcinoma of the pancreas is described and three similar cases reported in the literature are reviewed.

Statistical evidence is produced that this association is not fortuitous and theories of its causation are discussed.

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AMYOPLASIA CONGENITA

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(PLATES CXXVII AND CXXVIII)

AMYOPLASIA congenita is a name given by Sheldon (1932) to a rare congenital condition of rigidity of joints associated with small muscles. Rocher (1913), who first recognised the condition as an entity, described 31 cases from the literature and his own practice under the name of multiple congenital articular rigidities. It was called arthrogryphosis multiplex congenita by Stern (1923) and myodystrophia congenita deformans by Middleton (1934). Sheldon's name is best since it is short, and the disease is primarily muscular and there is a deficiency of formation fibres in number and in some cases one or more muscles appear to be absent macroscopically.

The condition is recognisable at birth by deformities and rigid joints. In most cases the disease is generalised in that it affects all limbs, but Rocher described cases with limitation of rigidity to one joint, two homologous joints or the joints of one limb or a pair. Many or all the joints of the limbs in the generalised form are fixed in flexion or extension, abduction or adduction, or in rotation according to the type of joint, and movement is absent or considerably restricted. In some cases the deformities are symmetrical, in others haphazard. Club hand and foot are present in most of the generalised cases. Scoliosis is present in a few, due to affection of the erector spinæ. The muscles of the head appear to escape, with the exception of one case (Ealing, 1944) in which the temporo-maxillary joints were rigid. The affected parts of limbs are of reduced circumference which often makes the knees and elbows appear large and fusiform. In radiographs the muscular shadows are very small. Electrical responses in affected parts are weak or absent but a reaction of degeneration has not been observed. Congenital dislocation of the hips is commonly present and Stern described tibial subluxation in two cases. Associated abnormalities in other tissues are rare. Rocher and Ouary (1929-30) described absence of the sacrum and abnormalities in the ribs and lumbar vertebræ and Middleton (1934) found microcephaly in one case.

The disease does not appear to affect the prospect of life, but in reported cases most of the subjects were infants or children and the oldest was fifteen (Magnus, 1902-03). In the cases collected by Rocher, males were affected about twice as frequently as females. A hereditary or familial incidence has not been recorded. In the obstetric histories oligo-amnios was described in three cases by Rocher and one by Price (1933) and hydramnios in two cases by Rocher and one by Ealing. Transverse presentation occurred in Ealing's cases and breech delivery in Sheldon's case and mine.

A case of amyoplasia congenita

For comparison with the subject of the case, an apparently normal female fœtus of 1220 g. wt. and 36 cm. crown-heel length was used as a control, as the size of the face, length of the limbs and the bony development as seen in radiographs were identical with those of the subject.

· *Obstetric history.* Spontaneous onset of labour in the thirtieth week of pregnancy in a primipara was followed by breech delivery of a stillborn fœtus, the subject of the case report.

Macroscopic examination. The specimen was a female fœtus of 1014 g. wt. and 39·5 cm. crown-heel length. There was marked bilateral talipes calcaneus and the neck was very short due to elevation of the scapulæ. Each index finger was flexed and each thumb flexed and adducted over the palm and very little extensor movement was possible. Movement of limbs, especially of the knees and elbows, was very limited and the legs and forearms were fixed in about 90° of flexion, extension obviously being impossible without rupturing the skin and, in the partly dissected elbow, without section of the anterior articular capsule. There was marked scoliosis extending from the mid-thoracic region to the mid-lumbar, the concave aspect facing the right and the bending centred at the level of the ninth thoracic vertebra. The uppermost two ribs on each side, the lowermost two left ribs and the posterior parts of the remaining ribs were normal, while the remaining parts of the ribs were very soft, thin and straightened. Bones of the limbs, spinal column and base of the skull appeared to be normal in radiographs except for the scoliosis. The musculature was examined in almost all regions, excluding the hands, feet and face, and everywhere appeared to be considerably reduced in size (figs. 1 and 2). In a dissection of the back of one forearm no trace was found of the extensor pollicis longus, extensor indicis proprius and extensor pollicis brevis and their tendons. Muscles were from a sixth to a third of their normal size as shown by the weights of the following muscles (weights in control fœtus in brackets): biceps brachii 0·475 g. (1·345); deltoid 0·715 (3·81); infraspinatus 0·45 (2·705); adductor pollicis longus 0·105 (0·37); extensor carpi ulnaris 0·18 (0·66); extensor digitorum communis 0·22 (0·9); extensor carpi radialis longior and brevior 0·315 (1·005).

There were a few petechiæ in the ventricular pericardium and the endocardium of the left side of the interventricular septum showed a faint streak of hæmorrhage. The cusps of the mitral valve contained four corpora albini and one of the tricuspid valve contained one corpus. The lungs were atelectatic. The liver, spleen, kidneys, thyroid, pancreas, bladder, meninges, skin, intestinal mucosa and salivary glands were congested. There was slight internal hydrocephalus of unexplained cause. There was no centre of ossification in the lower end of the femur. The larger peripheral nerves of the limbs were equal in size and appearance to those of the control fœtus.

Plate CXXVII

AMYOPLASIA CONGENITA

Fig. 1.—Amyoplasia congenita. Transverse section through middle of arm. Great reduction in muscular tissue with relative increase of adipose tissue. Weigert's iron hæmatoxylin and van Gieson. ×3·75.
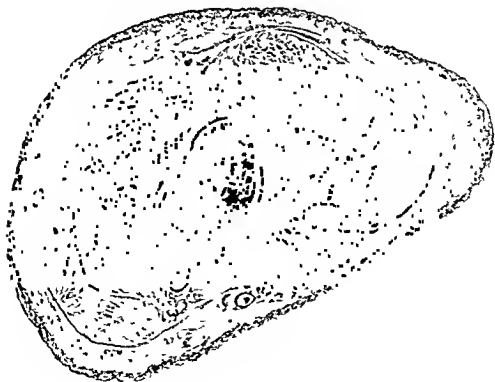
Fig. 2.—Normal fœtus. Transverse section through middle of arm. Weigert's iron hæmatoxylin and van Gieson. ×3·75.

Microscopic examination. The biceps, infraspinatus, deltoid, subscapularis, extensor digitorum communis, extensor carpi radialis longior and brevior and adductor longus pollicis were examined individually in transverse or longitudinal section. All muscles of the arm and leg were seen in transverse sections through the middle of the arm and junction of middle and lower thirds of the leg. Various intercostal muscles, muscles attached to various bones, especially at the knee, and the erector spinæ at the level of the fifth cervical and tenth thoracic vertebræ were examined. Sections of muscles were stained by Mallory's phosphotungstic acid hæmatoxylin and Heidenhain's iron hæmatoxylin besides hæmatoxylin and eosin or van Gieson's mixture. The most conspicuous abnormality was smallness of all skeletal muscles due to deficiency in the number of fibres in varying degree. The erector spinæ was affected most severely, and in the section at the level of the tenth thoracic vertebra, its usual site was occupied by adipose tissue. On one side two fibrous septa in the adipose tissue extended from the region of the spinous process to the anterior extremity of the lamina and contained two or three muscle fibres. In a more lateral fibrous septum in the superficial part of the adipose tissue on each side there was a group of two or three dozen muscle fibres. This musculature alone represented the erector spinæ in this section. In no section was it possible to show complete absence of a muscle. In transverse sections through the leg and arm not all muscles were identified, but in view of the minuteness of the erector spinæ it is quite possible that muscles which appeared to be absent macroscopically might have been represented by a few fibres only. The muscles showed abnormally small and few bundles of fibres. There was a slight but definite increase of the collagenous fibres of the endomysium and perimysium. In no muscle was there infiltration with adipose tissue. The adipose tissue occupying the site of the erector spinæ was probably formed between the individual components of the muscle, which can be regarded as separate muscles, and not within any one component. The most conspicuous abnormality in the fibres was a considerably increased variation in diameter (figs. 3 and 4). The diameter varied from 3 to 24 μ compared with 4·5 to 7·5 μ in the control foetus. This was due chiefly to hypertrophy of many fibres, in part to abnormal smallness of others. The length of fibres was not estimated. Longitudinal sections showed that most fibres were abnormal. Abnormalities were of four types. Firstly, in places in some fibres the discs were jumbled up, with loss of regular relationship between the discs in any one fibrilla or between those in adjacent fibrillæ. This occurred at the sites of acute kinking of fibres. In the control muscles, fibres in many places were slightly wavy, the waves having a relatively long wave length and a low amplitude. Similar waviness was present in the diseased muscles, but in addition kinks or sharp waves of short length were present in places. This jumbling was most probably produced after death

by rigor mortis or indirect injury, but it possibly indicates a lowered rigidity of the ground substance of the fibres. In longitudinal sections of muscles where fibres were cut across at the surface of the section, fibrillæ were very commonly frayed and disarranged in the diseased muscles and much less frequently in the normal control muscles. In the second type of abnormality there was diminution or loss of staining of the A and Z discs in part or the whole of the segments of a few scattered fibres present in the section. The affected parts were hyaline and were stained various shades of orange to deep blue in Mallory's phosphotungstic acid hæmatoxylin. A few similar hyaline fibres were found in control muscles, so that the condition is probably not abnormal. The third and most important abnormality affected most fibres but was absent from most of the much hypertrophied fibres. Whole segments or parts showed loss of A and Z discs in fibrillæ which were slender, less parallel than normally and stained homogeneously by Mallory's phosphotungstic acid hæmatoxylin. The change was not accompanied by hyalinisation. It appeared as if the substance of A discs had spread throughout the fibrillæ. Affected fibres stained very slightly or not at all with eosin. The fourth type of abnormality affected certain fibres showing abnormality of the third type. It consisted of granularity or vacuolation of the cytoplasm and a variable degree of diminution of fibrillar tissue which was absent in some affected fibres. Such fibres were usually very small and were seen scattered about in most fields. There was probably no absolute increase of muscle nuclei except in a few of the small fibres in which many nuclei were arranged in a chain along part of the fibre. The muscles were more cellular than those of the control fœtus, due chiefly to a relative increase in the number of muscle nuclei, but partly to an increase of connective tissue cells. Some of the hypertrophied fibres had central as well as peripheral nuclei. There was no definite increase of histiocytes, but one degenerated fibre was seen to be invaded by macrophages. Neuromuscular spindles appeared to be unaffected, but minor abnormalities, especially in the polar regions, could easily have been missed in single sections. Certainly the equatorial regions were well preserved. In transverse sections the spindles were more readily identified than in the control muscles. Nerve endings in the spindles and extra-fusal fibres were not stained by three different modifications of Bielschowsky's method in either the diseased or the control muscles, probably due to post-mortem changes. In transverse sections of muscles the abnormality of the structure of the fibres was much less conspicuous than in longitudinal sections, but the increased variation in diameter of the fibres was very conspicuous. In both normal and diseased muscles phosphotungstic acid hæmatoxylin stained the fibres in transverse sections almost homogeneously and the fibrillæ could only be distinguished with difficulty.

The articular surfaces seen in an acetabulum and glenoid fossa,

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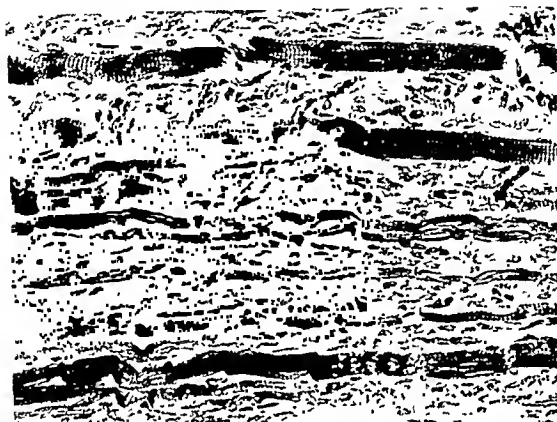
FIG. 3.—Amyoplasia congenita. Subscapularis. Hypertrophy and well preserved disc structure in some fibres and atrophy and abnormal disc structure in others. Mallory's phosphotungstic acid hæmatoxylin.   ×300.
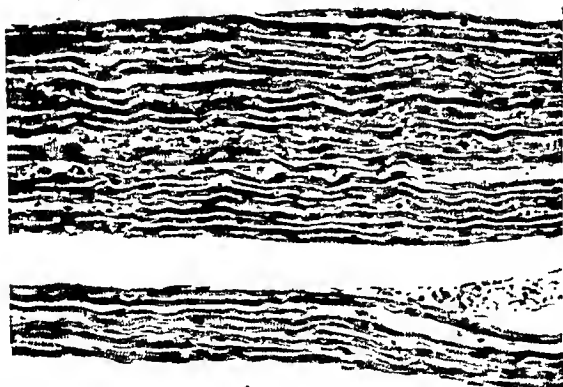
FIG. 4.—Normal fœtus. Infraspinatus. Mallory's phosphotungstic acid hæmatoxylin and van Gieson.   ×300.

on the head of a femur and tibia and in a complete median sagittal section of a knee appeared to be normal. In the knee the articular cavity was definitely smaller than in the control fœtus, but a slight difference in the plane of the sections might have accounted for this.

Bone was histologically normal in the upper and lower ends of a femur and upper end of a tibia, in coronal sections of the fifth cervical and tenth thoracic vertebræ and in median sagittal sections of the eleventh and twelfth thoracic and all lumbar and sacral vertebræ. Three left ribs with costal cartilages, the upper five right ribs and a group of five left ribs in vertical sections of the chest wall showed abnormalities. The three left costal cartilages in the 2 or 3 mm. adjacent to the bone were thin and hypoplastic, especially at an isthmus about 1 mm. from the bone, where the cartilage was 0·5 mm. broad or less. At this isthmus the perichondrium contained a cleft which probably surrounded the cartilage, since it was present on both sides in all three cartilages : it was probably an adventitious bursa. Endochondral ossification was normal but the osteochondral line was abnormally short, due to hypoplasia of the cartilage and 10-20 columns of hypertrophic cells only were present in sections across the hypertrophic zone. Endochondral bone in the ribs was replaced in those parts from 0·5 to 1·5 mm. beyond the cartilage by periosteal bone. All the ribs seen in transverse section consisted of thin plates of periosteal bone. *These changes were probably secondary to the deformity of the chest due to the scoliosis.*

The pituitary, external ear and musculo-spiral nerve, and the spinal cord at the level of the fifth and seventh cervical and ninth thoracic vertebræ were normal except for a definite reduction in number of ganglion cells in the anterior horns of the cord in the thoracic vertebral segment and reduction at least in average size of those in the seventh cervical segment. The sympathetic chain and a posterior root ganglion seen in sections at the level of the fifth cervical and tenth thoracic vertebræ were normal. No degeneration was seen in a few nerves of a brachial plexus in Marchi preparations.

Discussion

The interpretation of the histological changes in the voluntary musculature is difficult. The appearances suggest that the number of fibres in any muscle had always been deficient due to aplasia and that all or most of the fibres which had been formed became hypertrophied. The affection of the voluntary musculature appeared to be universal but variable in severity. Some muscles appeared to be absent macroscopically, but in view of the representation of the erector spinæ in a transverse section by a few fibres only, it cannot be assumed that any muscle was completely absent. The most marked structural change in the fibres was probably atrophic and affected most fibres.

The A and Z discs disappeared and the fibrillæ stained throughout with phosphotungstic acid hæmatoxylin. Since many of the affected fibres were larger than normal it is probable that the change began in fibres that were hypertrophied and possibly of previously normal structure. The fibres were probably shrinking in size. The atrophic change was complicated in some fibres by vacuolation and granularity of the cytoplasm and destruction of the fibrillæ. This change appeared to be degenerative and was probably leading to destruction of some of the fibres. It was highly unlikely that the smallness of the muscles was due primarily to the atrophic or degenerative changes.

References to the appearances of the muscles in the literature are few. Rocher at operations found the muscles pale and small and they retracted but little when cut. Magnus found no muscular tissue in the popliteal space in a subject of 15 years. Price in an infant of 5 months found small muscles, and the extensor carpi radialis brevis appeared macroscopically to be absent. Microscopically there were zones of atrophied fibres containing chains of nuclei and areas where muscle had been replaced by fibrous or adipose tissue. Middleton (1934), in a biopsy from a child of 7, found the muscle replaced by adipose tissue containing fibrous strands and remnants of two muscle fibres. In a biopsy from a child of 6 the muscle consisted of adipose tissue surrounding a core of muscle fibres. In an infant of 3 weeks a biopsy showed areas of normal fibres separated by areas of adipose and connective tissue. He noted that the neuro-muscular spindles were relatively easily demonstrated. It appears, therefore, that fibrosis and adiposity complicate loss of muscle fibres in amyoplasia congenita as in other muscular dystrophies.

The fixation of the joints was probably for the most part secondary to lack of voluntary and passive movements at the time the joints were being formed. This lack of movement would be expected to lead to deficiency in size of the articular surfaces, with attachments of synovia and capsules nearer the centre of the joint than normally, and these structures would probably be shorter and less movable and elastic than normally. The periarticular tissues, including the skin, would likewise be expected to be formed to cover a rigid tissue without the extra amount of tissue which would be needed to allow free movement at the joint. Sections in my case did not show any definite abnormality in the size of the articular cavities, but macro-scopically it was obvious that extension of the knee and elbow could not have been possible without section of the popliteal skin on the one hand and anterior capsule of the elbow on the other. It is likely that the slight fibrosis of the muscles in my case played a part in producing rigidities. Rocher believed the articular rigidities were due to such abnormalities of disposition of synovia in articular attachments, but also in part to retraction of muscles, fascia and aponeuroses. He found at operation that a knee could not be extended because of tightness of the popliteal skin and a plastic

operation had to be performed. Price, in an infant of 5 months, found that the articular surface of the lower end of the humerus did not extend on to the front of the bone and that on the trochlea and capitellum was confined to the inferior surface. The upper part of the great sigmoid cavity of the ulna had cartilage replaced by fibrous tissue which formed a band attached to the olecranon fossa of the humerus just above the margin of the trochlea. Flexion of the joint could be performed only when this band was cut. A band of fibrous tissue, apparently the orbicular ligament, passing from the external condyle of the humerus to the lower border of the lesser sigmoid notch tightened on flexion and appeared to limit movement. In the wrist the interosseus ligament between the semilunar and cuneiform bones was attached to the articular surface of the radius. Magnus, in a resected knee of a subject of 15, found hyaline cartilage on the femur only where there was contact with the tibia; the remainder had been replaced by connective tissue. This condition is likely to be metaplastic, due to prolonged disuse of articular cartilage, and would not of itself lead to rigidity unless there was union of the connective tissues of opposing surfaces. In a congenitally deformed dwarf, aged 18, with mongoloid face, great kyphoscoliosis and deformed ears, I found segments of the articular surface of a femoral head replaced by fibrous tissue, metaplasia of cartilage of a femoral condyle into dense fibrous tissue, fibrous tissue containing a few cartilage cells or typical fibrocartilage, and a similar change in the articular cartilage of the upper end of a tibia. The change in the cartilage was probably secondary to prolonged immobilisation of joints but the disease was not amyoplasia congenita: possibly it was dysplasia epiphysealis multiplex.

There is no definite evidence as to the nature of the abnormality which causes the original aplasia and the atrophy and degeneration of fibres already formed. It is possibly an intrinsic genetic abnormality lying in the muscle cells and not dependent upon external influences. More likely it is an extrinsic abnormality, probably of the motor nerve endings in the muscle. Voluntary muscle is not dependent for its formation upon the presence of nerves and it forms in exogastrulæ in the complete absence of nerve tissue (Huxley and De Beer, 1934), but in exogastrulæ it does not contract or develop fully and soon degenerates. A deficiency or degeneration of motor end-plates during the early development of voluntary muscle might therefore be expected to lead to an initial deficiency in number of fibres. Fibres which had normal end-plates would undergo compensatory hypertrophy, but if degeneration subsequently affected many of the plates atrophy and degeneration would follow. The muscular changes described in cats following section of ventral roots by Tower (1932) were not unlike those in my case. The extrafusal fibres showed faded cross striations, emphasised longitudinal fibrillæ and reduction in size, followed by destruction by vesicular degeneration. These

changes appear to correspond to the atrophic and degenerative changes seen in my case. Tower found that section of the ventral roots caused atrophy and degeneration of the intrafusal fibres only in the polar regions, that section of the dorsal roots caused degeneration in the equatorial region of the spindles after some months, and that section of peripheral nerves led to degeneration throughout the spindles. If the muscular disease in my case were due to a nervous abnormality, the apparent normality of the spindles, at least in their equatorial regions, would indicate that the abnormality was motor only. Degeneration of the motor plates would be expected to lead to degeneration, atrophy or loss of ganglion cells in the anterior horns of the spinal cord, varying in degree with the extent and duration of destruction of end-plates. The finding in my case of a reduced number of anterior horn cells at the level of the ninth thoracic vertebra and at least a reduced average size at the level of the seventh cervical vertebra supports the supposition that there is degeneration of end-plates. Among reported cases, only that of Price contains a description of the spinal cord. There was shrinkage and degeneration of neurons but there were other changes, many or all of which might have been due to post-mortem injury or prolonged fixation. The failure to stain end-plates in my case cannot be regarded as significant, since similar failure, due probably to post-mortem changes, occurred also with muscles of the control foetus.

It remains to discuss the relationship of the muscular dystrophy of amyoplasia congenita to that in the more common and better recognised condition, generally called progressive muscular dystrophy. Many types of this disease are known, but they fall into two groups, the first consisting of myatonia congenita and the Werdnig-Hoffmann disease, the second of the juvenile and pseudo-hypertrophic types.

In the first group, dystrophy is present at birth or appears during the first year and is shown by weakness and flaccidity of the limb muscles. The trunk, face and tongue may be affected but the diaphragm is generally spared. In myatonia congenita the dystrophy is congenital, familial incidence is usually absent, and the weakness tends to remain stationary, lessen or disappear, although in most cases some weakness persists. In the Werdnig-Hoffmann type the dystrophy is not congenital but appears in the first year, a familial incidence is usually present and the weakness is progressive and eventually leads to death, but periods of standstill or amelioration of weakness might occur. Contractures occur rarely, chiefly in myatonia congenita, but are not congenital. The two types are not sharply defined and in some cases, such as those of Krabbe (1920), features of both types are present. As shown by Greenfield and Stern (1927) there are no pathological differences between the types. Descriptions of the muscular changes differ, depending on the different stages of the disease at the time of observation, and probably also on differences in description of similar appearances. From the descriptions

of Councilman and Dunn (1911), Faber (1917), Holmes (1920), Greenfield and Stern (1927) and Lewey (1942), the chief changes are as follows. The muscles are abnormally small, soft, grey and translucent, or like raw pork. Microscopically there are bundles of small fibres, bundles of large fibres or bundles of both small and large fibres. Small fibres might be grouped in areas which are well defined from those with large fibres. Hypertrophied fibres are described by some authors. The small fibres have been regarded as atrophied by some authors and as underdeveloped or embryonic by others. Small-fibred areas are abnormally cellular, due to an increase of muscle nuclei which has been reported by some as only relative. Lewey described slender longitudinal fibrillæ without cross striation in the small fibres and Councilman and Dunn stated that the fibrillæ were not so parallel as normally and the distance between the Z discs was increased but cross striation was preserved. J. B. Holmes (1920) described a curved, sinuous or spiral form of the fibres and fraying of their ends where cut across. Vacuolar degeneration of small fibres and fibrosis of bundles of small fibres have been described. Adipose replacement of fibres which have disappeared occurs in late stages of the disease. Changes in the nervous system are present in most cases and are described by Greenfield and Stern and others. In most there is atrophy, degeneration and reduction in number of ganglion cells in the anterior horns of the spinal cord, and in some cases similar changes in medullary nuclei or atrophy or demyelination of the anterior spinal roots or peripheral nerves have been found. Foot (1913) and Bielschowsky (1929) found an absence of motor nerve terminals in the small atrophic fibres but their presence in large fibres. Tuthill and Levy (1931) found no end-plates in the tongue, which was affected in their case, but sensory nerve endings were well stained. Faber referred to other reports of absence of motor nerve endings in the muscles. There are no other significant pathological changes. Hypertrophy of the thymus or abnormal Hassall's corpuscles have been described in some cases mentioned by Councilman and Dunn and by Foot. From the available evidence there is nothing to indicate that the muscular dystrophies of amyoplasia congenita, myatonia congenita and the Werdnig-Hoffmann disease are not of similar ætiology and that the clinical and minor anatomical differences cannot be explained by differences in time, intensity and duration of application of a single ætiological factor. Also there is nothing to indicate that the primary abnormality might not lie in the motor nerve endings.

In the second group of muscular dystrophies—the juvenile and pseudohypertrophic forms—males are chiefly affected, the disease is often familial or hereditary and the onset occurs in childhood, adolescence or adult life. The hereditary transmission might be dominant, recessive or sex-linked. There is muscular weakness beginning symmetrically in different regions in different types of

cases, but eventually involving a large part or almost the whole of the voluntary musculature. Rarely, as described by Barnes (1932), weakness is preceded by hypertrophy and increased muscular power. The limbs, trunk and neck and the muscles of facial expression are most commonly affected, but the ocular muscles, tongue and masseters are involved in some cases. The weakness is accompanied by atrophy of the muscles, but atrophy in some muscles in the pseudohypertrophic form, especially in the calves and buttocks, is preceded by enlargement. Contractures occur in some cases. In general, there is a progressive loss of muscle fibres associated with replacement by adipose tissue. In some muscles replacement is complete. In general the muscles are abnormally small, but in the pseudohypertrophic form a few are enlarged even although there is extensive or complete replacement of muscular by adipose tissue. Fibres persisting in affected muscles are hypertrophied, normal in size or atrophied. Some fibres show vacuolar degeneration or hyaline coagulation of the sarcoplasm. Phagocytosis of degenerated or hyaline fibres may be seen. Hyaline coagulation is probably not a specific change but is most likely to be due to excessive contractile activity. Atrophied, degenerated or hyaline fibres often show proliferated muscle nuclei. In hypertrophied fibres central as well as peripheral nuclei are common. The neuro-muscular spindles in some cases have been found relatively unaffected. Fibrosis is common in bundles of atrophic or degenerated fibres, but apparently, after loss of the fibres, the fibrous tissue is replaced by adipose tissue. Reduction in the number of ganglion cells in the anterior horns of the spinal cord, and perhaps also atrophy of anterior spinal roots and peripheral nerves, have been observed in a number of cases, as described by G. Holmes (1908). In the available evidence there is nothing to indicate that the dystrophies in this second group are different in ætiology from those of the first, or that in amyoplasia congenita; many similarities suggest rather that the ætiology in each is essentially the same. It is likely that the ætiology in each is dependent upon a genetic abnormality, but the manner of transmission in each is different. In amyoplasia congenita there is no evidence of transmission of the factor; in the first group of dystrophies, comprising myatonia congenita and the Werdnig-Hoffmann disease, transmission is shown by a high familial incidence, while in the second group hereditary transmission is common.

The muscular disease in lambs described by Roberts (1929) closely resembles amyoplasia congenita, but in its hereditary transmission it resembles the progressive muscular dystrophies of the second group mentioned above. The condition is hereditary and dependent upon a homozygous condition of a recessive factor which is not sex-linked. There is hydramnios in the ewe shortly before the delivery of an affected lamb, which is almost always stillborn. The lambs show multiple articular rigidities due to retraction of greatly atrophied muscles. On removal of the muscles the joints are freely movable

but their capsules are slightly more tense than normally. The muscles, according to Middleton (1932, 1934), are composed of un-differentiated and adipose connective tissue containing small muscle fibres, which he likened to myoblasts, and few normal fibres. Muscles in premature lambs obtained by hysterectomy showed active degeneration of muscle fibres without adipose tissue. In two of three dissected lambs, according to Roberts, the thymus was thought to be hypertrophied.

Summary

A case of amyoplasia congenita is described and the relationship between this muscular dystrophy and those of myatonia congenita, the Werdnig-Hoffmann disease and the juvenile and pseudohypertrophic forms is discussed. It is concluded that the evidence supports the view that the diseases have a common ætiology.

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PRIMARY PULMONARY HYPERTENSION

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(PLATES CXXIX-CXXXVI)

PULMONARY hypertension is of two types. It is secondary when caused by certain cardiac and pulmonary disorders, including diseases of the pulmonary arteries such as syphilitic arteritis, which are dependent upon certain extrinsic factors. It is primary when these conditions are absent, and when vascular disease, if present, is not clearly dependent upon any extrinsic factor. Brenner (1935), reviewing 16 cases of primary pulmonary hypertension, concluded that there was no constant lesion in the arteries and that there were many problems in the disease which awaited solution. Experience in a recent case has enabled us to make a contribution to the pathology and pathogenesis of this condition.

CLINICAL FINDINGS

A woman of 44 years had experienced, for more than two years, great tiredness which prevented her from doing routine housework. She was also breathless, but a sense of fatigue was uppermost amongst her symptoms. Six months later her ankles and legs began to swell towards evening; her face was puffy in the morning, and it became mauve in colour on the least exertion including stooping. She had experienced one attack of sharp gripping pain across the upper part of the chest which lasted for only two minutes and there was no recurrence. There was also breathlessness at night, necessitating the use of four pillows. She was troubled with a cough but she never voided sputum. There was deep cyanosis of the lips and cheeks, and slight of the fingers, which were not clubbed. The blood count showed slight polycythæmia; the red cells numbered 5,900,000 per cmm., and the hæmoglobin content was 115 per cent. There was no breathlessness at rest when she was first examined. The pulse was very small, but normal in rate and rhythm. The blood pressure was 155/115 at six separate readings. The apex beat was quiet and displaced a little outwards. The heart sounds were clear and without murmurs and there was no triple rhythm on auscultation. The veins of the neck were distended and the venous column reached 2½ in. above the clavicle with the patient in the upright posture. Crepitations appeared at both lung bases. There was moderate distension of the liver and especially of its left lobe. There was no ascites. Œdema of the ankles disappeared at rest but some remained over the back. The urine always contained a small quantity of albumin; the blood urea was normal. The electrocardiogram (fig. 1) showed prominent right heart pre-

ponderance, for the S wave was deep in lead I, and the T wave was inverted in leads II, III and CR_1. On cardioscopy (fig. 2) there was great enlargement of the cardiac silhouette ; the enlargement involved the right auricle, the conus and body of the right ventricle and the pulmonary artery ; no enlargement of left auricle ; there was some congestion of the hilar vessels.

During the last nine months she was admitted to three different hospitals because of these symptoms, which were controlled by rest, digitalis and mercurial diuretics ; repeated injections of Neptal produced satisfactory diuresis on each occasion, but even though she appeared to be improving during her third period of residence in hospital, she died suddenly.

There had been some doubt about the clinical diagnosis. At the first hospital her condition was considered to be hypertensive heart failure ; at the second a diagnosis of pericardial disease was made, and on her third admission, although some pericardial effusion seemed to be present, the underlying enlargement of the right heart and of the pulmonary artery, together with the characteristic cardiographic changes, pointed to the diagnosis of pulmonary hypertension.

POST-MORTEM FINDINGS

Macroscopic appearances

Pericardial effusion (1 oz.). A few petechiæ in visceral pericardium. Heart (12 oz.) ; moderate hypertrophy and dilatation of right ventricle, which was 0·7 cm. thick (fig. 3) ; no hypertrophy of left ventricle (fig. 4) ; dilatation of right auricle ; two small adherent ante-mortem thrombi in right auricular appendage ; dilatation of tricuspid valve ring ; no enlargement of left auricle. Slight atheroma in aorta ; many small flecks of atheroma in pulmonary artery and its branches. Œdema and congestion of lungs ; calcareous nodule (0·15 cm., diameter) in left upper lobe. Calcareous nodule in two lymph glands under bifurcation of trachea. Severe reticular chronic passive congestion of liver (54 oz.). Firm congested spleen. Œdema and slight congestion of kidneys. Many petechiæ in injected peritoneum.

Microscopic appearances

Numerous arteries up to 3·5 mm. in external diameter were examined in nine portions of lung, two of which were cut in serial section. Another portion of lung included the distal end of the right branch of the pulmonary artery.

Medial changes

Deficiency of growth. In a large number of pulmonary arteries there were segments where the media was abnormally thin or absent, apparently from a developmental defect in growth. As many as ten such segments were counted in some sections and their total number throughout the lung was probably many thousands. Their distribution was uneven but could not be determined exactly, as the source of the blocks of tissue was not sufficiently well recorded. Where there

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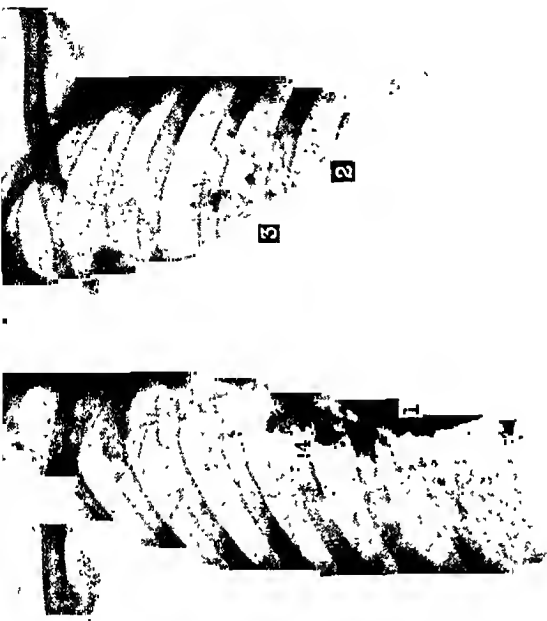
Fig. 2.—Teleradiogram showing enlargement of right auricle (1), conus of right ventricle (2) and pulmonary artery (3); moderate hilar congestion (4).
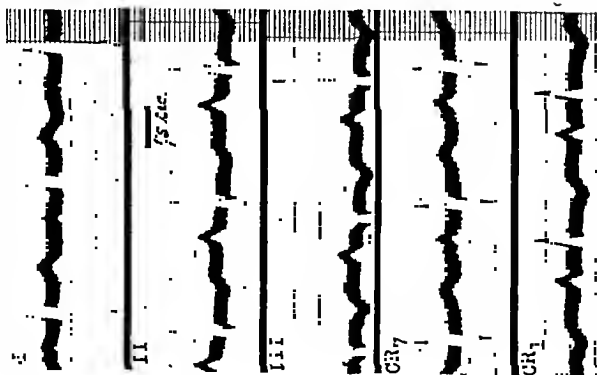
Fig. 1.—Electrocardiogram showing deep S wave in lead I and inversion of T waves in leads II, III and CR₇. T wave in CR₁ is upright.

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FIG. 3.—Right ventricle. Hart's elastic and van Gieson. ×7.
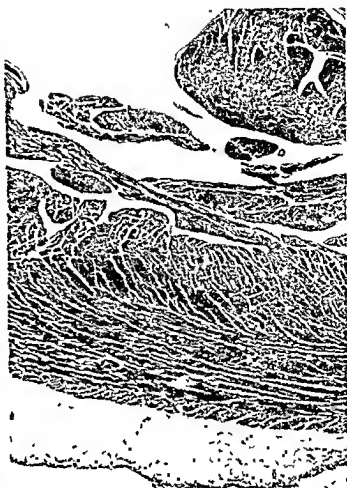
FIG. 4.—Left ventricle. Hart's elastic and van Gieson. ×7.

FIG. 5.—Distal end of right branch of pulmonary artery. Great hypoplasia of media and atheromatous endarteritis. Hart's elastic and van Gieson. ×125.

FIG. 6.—Distal end of right branch of pulmonary artery close to that in fig. 5. Hart's elastic and van Gieson. ×125.

FIG. 7.—Segment of medial aplasia at junction of elastic and muscular artery; slight intimal hypertrophy over segment. Verhoeff's elastic and van Gieson. ×130.

FIG. 8.—Medium-sized muscular artery and small muscular branch. Endarteritis fibrosa greatly stenosing small branch and surrounding its orifice. Medial degeneration in medium-sized artery recognisable as pale areas with disorganised structure. Aplasia or hypoplasia of whole of media of small branch readily recognisable on left close to origin of branch. Hart's elastic, Weigert's hæmatoxylin and van Gieson. ×115.

FIG. 9.—Focus of medial aplasia in large muscular artery. Verhoeff's elastic and van Gieson. ×290.

FIG. 10.—Small elastic artery. Medial hypoplasia on one side underlying great atheromatous endarteritis. Hart's elastic and van Gieson. ×85.

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FIG. 11.—Medium-sized muscular artery. Medial hypoplasia and aplasia; the former recognisable by approximation of lamellæ top left (10 o'clock). Crescent of endarteritis fibrosa. Hart's elastic and van Gieson. ×160.

FIG. 12.—Medium-sized muscular artery. Tangential cutting below, making media appear abnormally thick. Segment of hypoplasia upper left. Endarteritis fibrosa (right) containing a newly formed capillary which communicated with the original lumen at levels above and below that in the figure. Marked proliferation of endothelium of original lumen. Verhoeff's elastic and van Gieson. ×225.

FIG. 13.—Small muscular artery at junction with medium-sized artery. Aplasia or hypoplasia of whole of media of small artery; hypoplasia recognisable on left close to, and aplasia on right just below junction with larger artery. Canalised endarteritis fibrosa of small artery. Verhoeff's elastic and van Gieson. ×150.

FIG. 14.—Small muscular artery. Great stenosis of lumen by young granulation tissue containing a few lymphocytes and new capillaries. Replacement of endothelium of original lumen by layer of organising thrombus. Active medial degeneration to right, shown by œdema and irregularity of structures. Focal complete atrophy of media at about 5 and 10 o'clock. Verhoeff's elastic and van Gieson. ×225.

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Fig 11.

Fig. 12.

Fig. 13.

Fig. 14.

was aplasia the media was absent and the adventitia and intima were separated by only a single elastic membrane which split into internal and external lamellæ at the border of the affected area. Hypoplasia was shown by abnormal thinness of the media, which was of normal structure. The muscle cells in the hypoplastic parts were small. The aplasia was undoubtedly congenital. Hypoplasia might have been present at birth but post-fœtal growth of subnormal extent had occurred in the hypoplastic media.

The distal end of the right branch of the pulmonary artery showed a segment of hypoplasia the extent of which could not be determined, as the segment reached the edge of the section. The media was 60 μ thick (fig. 5) compared with 380 μ in the non-hypoplastic part (fig. 6), but the structure was normal except for increased compactness.

In arteries within the lung, segments of aplasia were common at the junction of elastic and muscular arteries (fig. 7) or at the branching of muscular arteries (fig. 8), and were less common in muscular arteries independent of branching (fig. 9). Segments of aplasia were usually small and seldom included the whole circumference of a vessel. In most examples the internal lamella was deflected outwards to fuse with the external lamella, and the deflection was gradual, so that aplastic areas were bordered by zones of hypoplasia (fig. 7). In two vessels the segments were very short and both lamellæ were sharply deflected towards each other (fig. 9). Segments of hypoplasia were rare in elastic arteries (fig. 10) but common in muscular arteries (figs. 11 and 12) and unrelated to branching, but, at the site of branching, hypoplasia could be recognised only when bordering on areas of aplasia. The whole of the media of some of the smallest muscular arteries appeared to be aplastic or hypoplastic (figs. 8 and 13). These vessels could be identified as arteries by the offset of arterioles from them and by the size of the arteries from which they took origin.

Atrophy. In many small and medium-sized muscular arteries, thinning of the media appeared to be due to loss or reduction of muscle fibres. The criteria for determining whether thinness of a media was due to deficiency of growth or atrophy, however, were not clear. Indeed, much medial thinning was seen in small muscular arteries which we did not classify as either atrophic or hypoplastic since, although the preservation of a normal structure suggested hypoplasia, dropping out of a few muscle fibres might have been the cause. Complete atrophy was shown by apposition without fusion of the internal and external lamellæ. In partial atrophy the lamellæ were separated by a thin layer of hyaline collagen only, or by a less fibrotic media containing irregularly arranged muscle fibres in reduced number. Atrophy was usually focal and accounted for the variation in medial thickness in figs. 14-16 ; in a few small muscular arteries it involved the whole circumference of the vessel.

Rupture. In one elastic artery the media was interrupted by a gap, 120 μ wide, occupied by collagen (fig. 17). The appearances

suggested a healed rupture. Small ruptures of the internal elastic lamella were seen in a few muscular or elastic arteries in any section, but in most vessels the lamella was intact. A few elastic or large muscular arteries showed small healed ruptures involving the internal lamella and a part of the subjacent media, which was replaced by collagen and small spindle cells (fig. 18). A recent rupture was seen in a large muscular artery. A segment 80 μ long of the media was replaced by a patch of reticulated fibrin which contained a few lymphocytes and proliferated fibroblasts and which extended into the adventitia.

Degeneration. In several muscular arteries the media showed vacuolation of muscle cells, apparent loss of some cells, and irregular arrangement of the remainder due to intercellular œdema (figs. 8, 14 and 19). The vacuolation was probably due to fatty degeneration, but this could not be proved, as tissue had not been reserved for the preparation of frozen sections. In a small elastic artery there was a patch of medial atheroma subjacent to intimal atheroma and shown by a patch of collagen containing necrosed lipoid phagocytes beneath the internal lamella.

Hypertrophy. Increased thickness of the media was apparent in many muscular (fig. 20) and elastic arteries. A media of 260 μ thick in a small elastic artery of 1·6 mm. external diameter, and a media of 48 μ thick in a muscular artery of 280 μ diameter, are examples of hypertrophy. The muscle cells were hypertrophied and well separated from each other by an increased amount of fluid ground substance. Only in small foci in a few vessels was there evidence of hyperplasia of muscle fibres. It was seen chiefly as groups of small longitudinal fibres under the internal or external lamella and usually separated from the remainder of the media by an elastic layer continuous with the lamella.

Intimal changes

Hypertrophy. Intimal thickening which could be interpreted as purely hypertrophic was seen in a few elastic and in several muscular arteries, but it was always slight. It was generally focal, but in a few smaller muscular arteries it involved the whole circumference. In the elastic arteries this intima consisted of collagen fibres, numerous very delicate elastic fibres, and a few muscle cells and fibroblasts. Duplication of the elastic lamella was slight or absent and there was no definite elastic stripe or longitudinal and circular layers of fibres. In one vessel there was a crescent of intimal hypertrophy in which muscle cells and fine elastic fibres were numerous (fig. 21) and which was sharply defined from the atheromatous thickening of the adjacent and overlying intima. In muscular arteries definite hypertrophy was observed as a thickening which consisted of delicate collagen and elastic fibres enclosing relatively numerous muscle cells without formation of layers.

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FIG 15—Small muscular artery Lamellæ crenated and media focally atrophied Lumen obliterated by a fibrous mass Hart's elastic, Weigert's hæmatoxylin and van Gieson ×340.

FIG 16—Small muscular artery with lumen obliterated by a fibrous mass, crenated lamellæ and focally atrophied media Respiratory bronchiole showing three dilated capillaries projecting into lumen Hart's elastic and van Gieson ×200

FIG 17—Small elastic artery. Complete interruption of media from old rupture occupied by fibrous tissue Hart's elastic, Weigert's hæmatoxylin and van Gieson ×100

FIG 18—Junction of elastic and muscular artery. Small rupture of internal elastic lamellæ at junction Media underlying ruptured lamella also ruptured, and replaced by collagenous tissue. Atheromatous intima to left Verhoeff'e elastic and van Gieson ×270

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FIG 19—Small muscular artery Endarteritis fibrosa overlying degenerated media, latter appearing as pale staining area The central part of the external elastic lamella was stained poorly and has been accentuated in figure by ink Verhoeff's elastic and van Gieson ×430

FIG 20—Muscular artery with medial hypertrophy. Verhoeff's elastic and van Gieson ×85

FIG 21—Small elastic artery Area of intimal hypertrophy on left and separated from overlying and adjacent atheromatous intima by elastic layer Verhoeff's elastic and van Gieson ×105

FIG 22—Small elastic artery Atheromatous endarteritis Small patch of acellular collagen near origin of muscular branch (lower right) Verhoeff's elastic and van Gieson ×16

Atheromatous endarteritis. The intima was thickened in all elastic arteries and varied from 40 to 680 μ thick. In only a few places was there evidence of hypertrophy. Elsewhere the thickening was atheromatous and consisted of collagenous tissue, fibroblasts, lipoid phagocytes and lymphocytes (figs. 6, 10, 21 and 22). In most but not in all places there were many and extremely delicate elastic fibres, and in a few places a few muscle cells were present. The phagocytes were scattered singly or in small groups and many were necrosed. In the debris of some of the necrosed cells minute crystal clefts could be seen. There were minute spaces from which phagocytes had disappeared. Such spaces were common in patches of acellular collagen up to 500×125 μ (fig. 22). There were no areas of necrosis similar to those in atheroma in systemic arteries, where digested extracellular tissue is included in the mass. Autolysis had involved necrosed cells only. Atheroma was seen in only two of the muscular arteries and in these the greatly thickened intima was composed almost entirely of lipoid phagocytes.

Hypertensive endarteritis. In two small muscular arteries the endothelium had disappeared and the lumen was occupied by a homogeneous and almost clear coagulum containing a few lymphocytes and small spindle cells (fig. 23). Proximally the lumen was stenosed by fibrotic tissue and distally it was occluded by almost acellular collagen, which was œdematous, especially at the centre of the vessel. In several other small muscular arteries the lumen was stenosed by very œdematous tissue consisting of collagen fibres and few fibroblasts. In these vessels transition was seen between this œdematous tissue and densely fibrotic tissue in adjacent parts. Numerous muscular arteries, especially the small ones (figs. 8, 11 and 12), and a few arterioles (fig. 24), showed focal or concentric fibrotic intimal thickening. Delicate elastic fibres or a few muscle fibres were present in some of these intimas, but they had obviously appeared secondarily and did not indicate that hypertrophy had preceded the fibrosis. Many small muscular arteries were occluded by a dense collagenous mass lying internal to the internal elastic lamella (figs. 15 and 16). In most masses there were few fibroblasts and in some masses there were a few lymphocytes, muscle cells, elastic fibres or soot granules. The elastic lamellæ were often crenated from contraction of the vessel. A few of the occluded vessels which were infiltrated with lymphocytes appeared to be disintegrating. The sequence of events in this endarteritis appeared to be an infiltration of the intima with a fluid which became organised, and subsequently densely collagenous. The amount of this fluid probably varied greatly and it was great enough in a few vessels to obliterate the lumen. It is possible that some of the endarteritis fibrosa began as a proliferation of fibroblasts without infiltration with fluid.

In a few muscular arteries active endarteritis had a different appearance. The lumen was greatly stenosed by a thickened intima

consisting of young œdematous granulation tissue containing plump proliferating fibroblasts, a few lymphocytes, and canalising or canalised groups of proliferated endothelial cells which had grown down from the endothelium of the vessel (fig. 14). In very œdematous parts there was a little fibrin in homogeneous or reticulated patches. In two of these vessels the surface endothelium had disappeared in part and was replaced by a thin zone of organising fibrinous clot.

Several fibrotic intimas in small muscular arteries contained one or more well-formed capillary spaces with or without a surrounding layer of a few concentrically placed fibroblasts and collagen fibres (figs. 12 and 13). In some vessels the canalisation was great in proportion to the amount of collagenous tissue. Rarely did a newly formed capillary pass through the media to join a neighbouring capillary. In general, capillaries in fibrotic intimas opened into the original lumen below as well as above an obstruction or obliteration due to endarteritis fibrosa; when the original lumen was replaced by several canals, as in fig. 13, these opened into separate arterioles.

Endothelial proliferation. Endothelial proliferation, apart from that described in association with endarteritis, was common, and conspicuous in some vessels. It most frequently affected arterioles dilated up to 475 μ diameter (figs. 25 and 26). Some of these vessels were almost filled with proliferated endothelial cells, which formed papillary masses projecting into the original lumen or lined newly formed capillary spaces. In one arteriole (fig. 25) solid masses of endothelial cells in two places penetrated the wall and one mass became continuous with the walls of adjacent and dilated capillaries. Capillaries arising from these arterioles were dilated up to 160 μ diameter (fig. 26). Endothelial proliferation was seen in several capillaries (fig. 27) and especially in those arising from similarly affected arterioles; rarely it affected small muscular arteries (fig. 12).

In several arterioles with proliferated endothelium, small rounded masses or diffuse patches of eosinophil homogeneous material were seen amongst, or closely invested by, endothelial cells, or projected as polypi into the lumen, covered or uncovered by endothelium. While the appearances suggested that the material was hyaline thrombus invaded or overgrown by endothelium, Mallory's phospho-tungstic acid hæmatoxylin showed that fibrin was absent from the masses or was present as streaks or patches forming only a small part of the whole. It did not stain by Gram's method. It was comparable with the hyalin which is seen in afferent renal arterioles in hypertension, where Mallory's stain often reveals small patches of blue-staining fibrin-like material. Indeed, the hyaline material in a capillary affected by endothelial proliferation was continuous with that in an arteriole which was identical in appearance and distribution with that in systemic arterioles in hypertension. No other similarly affected arteriole was seen.

PLATE CXXXV

PRIMARY PULMONARY HYPERTENSION

Fig. 23.—Small muscular artery. Loss of endothelium and replacement of lumen by almost clear fluid containing a few lymphocytes and fibroblasts. Organisation of fluid leading to endarteritis fibrosa stenosing lumen on right. Verhoeff's elastic and van Gieson. ×285.

Fig. 24.—Great stenosis of arteriole by crescent of endarteritis fibrosa. Verhoeff's elastic and van Gieson. ×300.
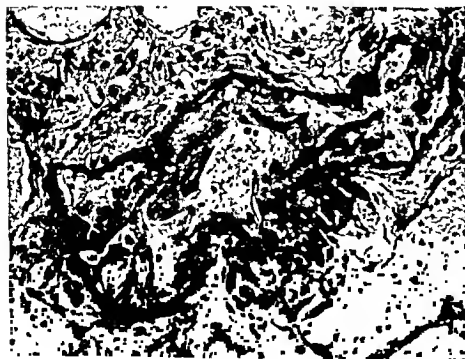
Fig. 25.—Arteriole showing proliferated endothelial cells with canalisation. Infiltration of endothelial cells through arteriolar wall into interstitial tissue at 4 o'clock. Verhoeff's elastic and van Gieson. ×240.

PRIMARY PULMONARY HYPERTENSION
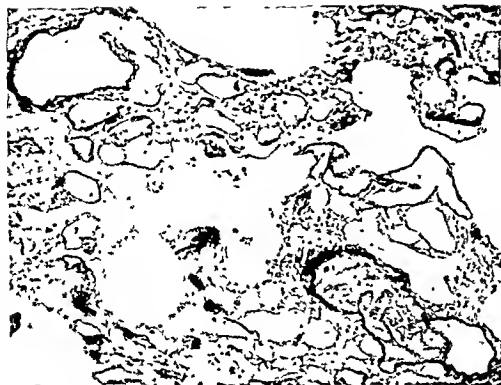
FIG. 26 —Two dilated arterioles, one at 10 and another at 4 o'clock, separated by alveolar passage. Canalised proliferated endothelium in lower arteriole Dilated capillaries projecting into alveolar passage. Hart's elastic and van Gieson. ×86.

FIG. 27.—Canalised proliferated endothelium in dilated capillaries arising from arteriole in fig 24 Hart's elastic, Weigert's hæmatoxylin and van Gieson. ×240.

FIG. 28 —Medium sized muscular artery. Segment of medial aplasia extending from A to B Great endarteritis fibrosa. Capillary vessel passing through aplastic wall causing anastomosis between artery and neighbouring dilated capillary. Branch of capillary lying upon elastic of aplastic media close to arrow at B. Hart's elastic, Weigert's hæmatoxylin and van Gieson. ×140.

Other changes

The course of an unusual vessel shown in fig. 28 was impossible to trace, as the block was not cut in series. It was a medium-sized artery cut longitudinally and stenosed considerably by endarteritis fibrosa. There was a long segment of medial aplasia extending from A to B in the figure. A wide capillary vessel extended from the arterial lumen through the aplastic arterial wall to join a neighbouring dilated capillary, a branch of which abutted directly upon the elastic membrane representing the aplastic media. This anastomosis was probably produced to relieve obstruction due to endarteritis and therefore resembled the anastomoses between arteries and neighbouring capillaries described earlier.

A bronchiole was greatly stenosed by a laminated crescent of collagen fibres and fibroblasts lying between the epithelium and the muscle, and its deeper part contained a few capillaries. The crescent probably resulted from organisation of fibrinous exudate in the lumen. Nearby in the same section there was a miliary fibrous nodule containing fibroblasts and the collapsed crenated elastic framework of alveoli. The nodule had resulted from organisation of pneumonic exudate. A few bronchi showed slight chronic inflammatory changes and slight hyaline thickening of the basement membrane of the surface epithelium. There were small patches of collapse and slight emphysematous dilatation of many scattered alveoli. In scattered small patches the alveoli were filled with coagulated œdema fluid. The lung tissue generally was slightly congested, and greatly around arterioles showing dilatation or endothelial proliferation distal to the stenosed or occluded arteries. Considerably dilated capillaries were seen projecting as polypi into the air passages (figs. 16 and 26).

Relationship of the changes

By far the greatest atheromatous endarteritis in elastic arteries was associated with medial hypoplasia (figs. 5 and 10). At the junction of elastic and muscular arteries, or in muscular arteries, medial aplasia or hypoplasia was associated with either no other abnormality (fig. 9) or slight hypertrophy of the intima (fig. 7), or hypertensive endarteritis (figs. 8, 11, 13 and 28). Almost all the small muscular arteries with medial deficiency showed endarteritis. In vessels with medial degeneration or atrophy there was almost always endarteritis in an early or fibrotic stage (figs. 8, 14, 15, 16 and 19). Atrophy appeared to be the late result of degeneration. The association of hypertensive endarteritis with either medial deficiency or medial degeneration or atrophy suggested that degeneration tended to affect the hypoplastic media particularly. This was possibly true, and the combination of degeneration and hypoplasia was seen in a few vessels although its recognition was very difficult, and degeneration

was clearly recognisable only in media of normal thickness. Medial ruptures did not show any consistent associated change, but the single recent rupture affected a focally hypoplastic degenerated media with overlying active endarteritis. Intimal or medial hypertrophy showed no constant or significant associated change. Atheromatous endarteritis did not appear to have started in the hypertrophied intima. Fine elastic fibres in most places and a few muscle fibres in some places were probably late in appearance and were not an indication of pre-existing hypertrophy. Endothelial proliferation formed new vessels relieving obstruction caused by endarteritis; it occurred in the thickened intima itself or in the arterioles or capillaries distal to the obstruction. In the thickened intima the new vessels passed through the wall of the artery to form a collateral circulation, or caused a communication of the lumen above the obstruction with that below, or a communication of the lumen of a stenosed artery with an obstructed branch.

DISCUSSION

In the literature the cause of pulmonary hypertension has usually been ascribed to either thrombosis, atheroma or endarteritis. In our case the last two changes were very marked in the lungs. Before discussing the significance of these changes in the pulmonary vessels we give our conception of the nature of similar changes in systemic arteries. We regard atheroma as an infiltration by lipoid substances of the extracellular fluids of certain tissues due to a defect in metabolism inherent in man. The hypercholesterolaemic form of primary essential xanthomatosis (Thannhauser, 1940) is possibly an accentuated form of this defect. Most of the lipoid consists of cholesterol esters and much is taken up by phagocytes. Necrosis commonly supervenes and is of the coagulative kind, often proceeding to such a degree that the tissue becomes a homogeneous structureless mass of protein, after which the process subsides or ceases. The necrosis is a type which can be called atheromatous, since it is characterised by the presence of free cholesterol crystals and much fatty substance, largely soaps, in the protein. Free fatty acids, particularly oleic acid, liberated by splitting of cholesterol esters, are the probable cause of the necrosis. The free cholesterol crystallises out and the fatty acids are largely saponified. Inflammation occurs around the necrosed tissue and is seen as infiltration with lymphocytes and leucocytes, and proliferation of fibroblasts and sometimes of capillaries as well. Later the necrosed tissue often becomes enclosed in hyaline collagen. Atheroma affects the dense fibrous tissue of the septum fibrosum and valve rings and cusps of the heart in addition to the arteries. Fibrosis of the cusps or intimal hypertrophy (Turnbull, 1914-15) in arteries increases the susceptibility to atheroma and there is some evidence that strain independent of these changes also increases it. In arteries,

atheroma begins in the intima but necrosis in some cases also affects the subjacent media. Either there is necrosis and dropping out of muscle fibres followed by atrophy of the media, or, less commonly, lipoid infiltration extends into the media where it is followed by atheromatous necrosis. Medial necrosis is important, since the ⋯ ⋯ ⋯ ⋯ ⋯ ⋯ ⋯ ⋯ ⋯ ds to cause intimal hypertrophy, ⋯position to atheroma. Hypertensive ⋯ies, chiefly renal, in a proportion of cases of severe hypertension. The first change is probably the appearance of fluid in the intima, causing a variable degree of thickening. In some examples the fluid is mucoid, in others it contains lipoid particles or cholesterol-ester phagocytes. Occasionally the fluid is absorbed, leaving an intima distended by lipoid phagocytes, but usually there is a proliferation of fibroblasts and the formation of collagen and later dense fibrosis. In the early stages of this organisation a few lipoid phagocytes and lymphocytes may be present. Fatty degeneration of the medial muscle fibres is often seen in the early, and medial atrophy in the later stages. The cause of this change is not apparent but it is related to hypertension and is possibly secondary to medial injury. The early stage must not be confused with the very common post-mortem entry of plasma into the intima of small arteries, which is related, not to hypertension, but possibly to vascular rigor mortis. Muscle and elastic fibres can appear secondarily in an intima which shows endarteritis fibrosa. This must not be confused with fibrotic intimal hypertrophy, although the function of the fibres and the stimulus which produces them may be the same as in a primary hypertrophy. Hypertrophy is an increase in the size of a tissue due to an increase in the size or number of its cells and of the extracellular structures which are normally formed by its cells. While endarteritis fibrosa is strictly a hypertrophy, since there is no evidence that its fibroblasts are not intimal in origin, we restrict the term hypertrophy to an increase in size to strengthen the vessel wall. Turnbull defined intimal hypertrophy as an increase in the magnitude of the intima with a structure similar to that in the normal aorta. We prefer to call this typical hypertrophy and to regard as atypical hypertrophy, thickened intima composed chiefly of muscle (Gilmour, 1941), or of muscle, collagen and elastic, without the specific organisation seen in the normal aortic intima. Intimal hypertrophy in pulmonary arteries is rarely typical.

In the pulmonary arteries in our case the appearance of these changes was slightly different from those seen in systemic vessels. Although atheroma was extensive, atheromatous necrosis was absent. Necrosis affected individual lipoid phagocytes, but there was never autolysis of extracellular tissue, and the collagenous framework persisted unaltered in the region of necrosed cells. There was, however, the formation of much fibrotic granulation tissue, and a

slight lymphocytic infiltration which we have called atheromatous endarteritis. We had no sections available to stain the fluid in the early stage of hypertensive endarteritis for fat and mucus, but the appearance in hæmatoxylin and eosin sections did not suggest mucus, and lipoid phagocytes were absent. In its association with medial degeneration and its organisation to produce endarteritis fibrosa it was similar to that described in systemic arteries.

Histological examination made it clear that the pulmonary hypertension was due to stenosis or blockage of muscular arteries by endarteritis fibrosa. Stenosis of elastic arteries by atheromatous endarteritis probably accentuated the hypertension, but it was not its immediate cause. The atheroma, we think, was the result of strain upon the arteries due to hypertension. Although hypoplastic segments might have been strained when the pressure was normal, leading to atheroma at these sites before the onset of hypertension, we do not think this atheroma could have been extensive enough to have been the cause of the hypertension. Probably the disease began as hypertensive endarteritis in muscular arteries at sites of aplasia or hypoplasia before the onset of persistent hypertension. The presence of a relative hypertension at such sites when the blood pressure was actually normal can be presumed, but transient hypertension might well occur, during coughing for example, and cause endarteritis. Once this endarteritis was extensive enough to produce permanent hypertension a vicious circle would be set up, since the strain would cause atheroma in elastic arteries and endarteritis in muscular arteries over normal-sized media, but more severely over aplastic or hypoplastic media. These changes would accentuate the hypertension so that the disease would become progressive. Therefore, the immediate cause of the hypertension was endarteritis which began because of the presence of very numerous medial deficiencies. Coughing or other cause of transient pulmonary hypertension might have started the condition on its course, but since it is not sufficiently distinctive by itself to merit the term disease, the hypertension is regarded as of the primary type.

We have seen medial deficiency in pulmonary arteries of several lungs in cases of secondary pulmonary hypertension as well as in cases without pulmonary hypertension. We think that such deficiencies are common, and probably constant, and that their number might play a very important part in determining the incidence of both primary and secondary pulmonary hypertension. Examination of other cases will decide whether the medial defects are the essential cause of primary hypertension.

The changes in the lungs in different cases are undoubtedly variable but this need not exclude a common ætiology. In a case of severe primary pulmonary hypertension of at least 40 years' duration we found very few medial deficiencies and the chief abnormalities were atheroma, rupture and thrombosis in small elastic and large muscular

artenes   The number of the smaller muscular arteries appeared to be considerably reduced   In this case endartentis and medial deficiencies might have been present early in the disease in smaller muscular arteries, causing subsequent absorption of these vessels by the obliterative process seen in figs 15 and 16   In the case described in this paper the duration of the disease was about two years and one would expect, therefore, to see early changes in the vessels

SUMMARY

Primary pulmonary hypertension in a case which is described was due to stenosis or blocking of muscular pulmonary arteries by endartentis fibrosa   The endartentis resembled that found in the systemic arteries in systemic hypertension and was also probably secondary to hypertension   Foci of medial aplasia or hypoplasia in the arteries numbered many thousands   We believe that endartentis occurred over some of these deficiencies during attacks of mild transient hypertension, such as might have been produced by cough ing , subsequently the resulting stenosis or occlusion led to persistent hypertension   The presence of this development deficiency in the media of innumerable small pulmonary arteries throughout the lungs is therefore regarded as an essential factor in the genesis of the endartentis and responsible for initiating the vicious circle which resulted in the progressive functional and structure disorder described in the present case

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SALIVARY ADENOMA AND ADENOLYMPHOMA

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(PLATES CXXXVII-CXLI)

THE adenolymphoma is one of the less common benign salivary-gland tumours and in this paper there is an account of a variety in which there is a considerable preponderance of the lymphoid tissue with the adenomatous elements relatively inconspicuous ; there is also a discussion of the much less frequent solid adenoma and its possible relationship to the adenolymphoma.

Adenolymphomata usually appear in the 5th, 6th or 7th decade and there is a male to female preponderance of five to one. They grow slowly, the duration of symptoms varying from a few months to thirty years. They are rounded, smooth or lobulated and measure from 1 to 4 cm. in diameter. They are usually fluctuant but may be hard, are attached to deeper structures but not to the skin, are encapsulated but not necessarily completely, and occur most frequently in the parotid gland, though occasionally in the submaxillary gland. Typically the tumour is a tubular or papillary columnar-cell adenoma with a greater or lesser degree of cyst formation ; the stroma is usually lymphoid but this characteristic may only be apparent in small areas. However the histological type varies considerably, as has been pointed out by Carmichael, Davie and Stewart (1935), who reviewed 34 cases including eight of their own. Thus the epithelial layer may be many cells thick (for example see fig. 20), or may show squamous metaplasia either throughout the tumour or mixed with tubular elements. The lymphoid tissue may be abundant, with many large germinal centres, or may be represented only by rare lymphocytic aggregates. Occasionally the tubular or papillary arrangement may be irregular and mitoses may be numerous but carcinomatous change is rare, only two cases having been reported. Sarcomatous change in the stroma does not yet seem to have been reported.

In 1942 Plaut tabulated 48 cases reported in the literature, with full references, and added 16 cases, bringing the total of reported cases up to 64. After describing his cases, Plaut mentions three others which, though clinically similar, differ somewhat in their histology from the usual types of cystic

adenolymphoma. " Like the usual form of adenolymphoma they contain a matrix of lymphoid tissue. In areas, this consists of diffuso lymphocytic infiltration. Elsewhere, there are aggregates resembling germinal centers. In the center of the latter are groups of large cells which resemble epithelioid cells rather than young lymphocytes. Here and there are many small cysts lined with low cylindrical cells with vesicular nuclei. Between the bases of these are small cuboidal cells, irregularly spaced. The cysts contain a palo pink, homogeneous material, cellular debris, and occasionally cholesterol crystals. In addition to the small cysts, and scattered in the lymphoid matrix, are other epithelial cells, singly and in strands. In places the tumor is separated from the parotid gland by a thin, fibrous capsule. Elsewhere, there is no capsule and the tumor infiltrates the glandular parenchyma ".

Fein (1940) describes a similar parotid tumour but calls it a lympho-epithelioma, though it shows no evidence of being carcinomatous.

In the present paper there is an account of seven cases of a solid variety of adenolymphoma resembling the three described by Plaut and that of Fein ; they were collected at the Radcliffe Infirmary, Oxford, between 1939 and 1945, for the most part through the agency of the Oxford lymph-node registry. Six of them were benign, while one showed sarcomatous change of the stroma. A brief clinical note and a detailed histological description of each tumour is given, but in general it may be said that they formed circumscribed firm masses of moderate size situated in a salivary gland and were readily excised from the surrounding glandular tissue. Histological examination showed that several of them were poorly encapsulated and consisted of a lymphoid stroma in which were lying widely separated epithelial elements ranging from solid acini of poorly differentiated epithelial cells to tubules and cysts lined by columnar epithelium ; it was not uncommon to find some lymphoid proliferation in the interstitium of the glandular tissue outside the capsule of the tumour.

Case reports

Case 1. *F.* 62 (*figs.* 1 *to* 5). A small tumour in the right parotid region was locally excised in 1939. It recurred and was again removed in 1940 and again in 1941, after which a course of deep X-ray therapy was given and there has been no further recurrence. Material was available for histology from the first and second biopsies. They resemble one another histologically. The stroma consists chiefly of lymphoid tissue with large ill-defined Flemming's centres and quite inconspicuous sinuses. Scattered throughout the lymphoid tissue are epithelial acini and tubules of salivary gland character. The tumour is bounded by a thin capsule of fibrous tissue, but this is incomplete and there is lymphoid infiltration of the salivary glandular tissue around it ; in this surrounding tissue the salivary acini and tubules show evidence of hyperplasia. Within the tumour some of the acini show squamous metaplasia (figs. 3 and 4) and some of the tubules are a little dilated but are still lined only by the usual double layer of epithelial cells, of which the superficial is columnar but not ciliated, the basal cubical, while others show multiplication of this basal layer and have become as many as five cells thick. There are no dilated tubules or cysts in the substance of the tumour removed at the second biopsy, but that first removed shows a number of cysts, sometimes lined by a double layer of epithelium, sometimes by a multicellular layer. Part of the wall of one of

SALIVARY ADENOMA AND ADENOLYMPHOMA

Fig 1.—Case 1. A solid acinus of epithelial cells near the epithelial lining of a cyst. ×115.
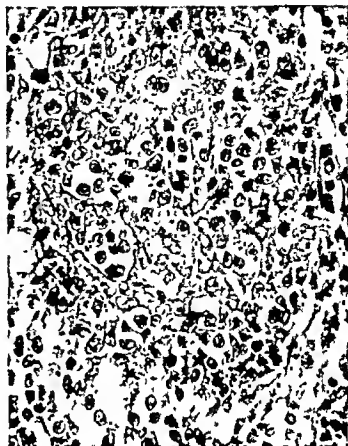
Fig 2.—Case 1. A solid acinus of epithelial cells. ×390.
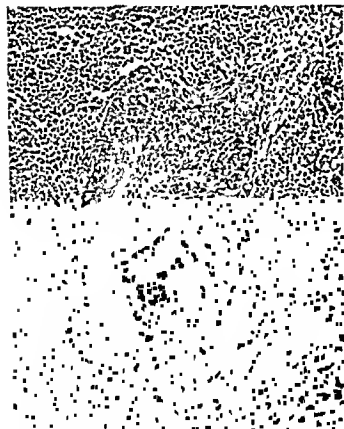
Fig 3.—Case 1. A hollow acinus of epithelial cells showing squamous metaplasia ×115.
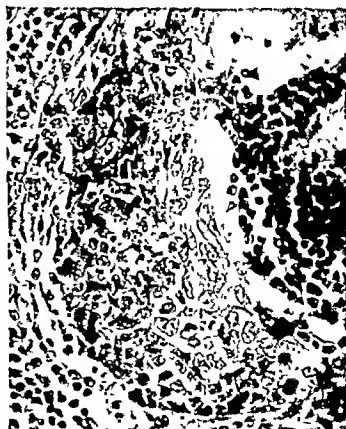
Fig 4.—Case 1. A higher-power view of part of fig. 3. ×315

SALIVARY ADENOMA AND ADENOLYMPHOMA

FIG. 5.—Case 1. The transition between tubules and solid acini, such as those seen in figs. 1 and 2. ×100.

FIG. 6.—Case 2. A solid acinus and several tubules set in a diffuse lymphoid stroma. ×115.

FIG. 7.—Case 3. Solid acini of squamoid epithelial cells set in a lympho-histiocytic stroma. ×135.
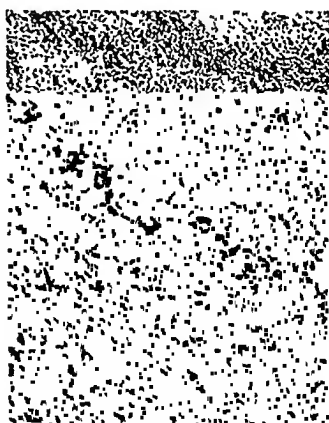
FIG. 8.—Case 4. Ill defined tubules set in a stroma which shows a lympho-histiocytic medullary proliferation. ×135.

the latter is shown in fig 1   In this biopsy, too, part of the stroma is fibrotic and shows early myxoid degeneration, particularly in relation to the tubular and cystic structures

In the second biopsy can be seen all grades of metaplasia from typical tubules, solid acini of epithelial cells (in a low power view rather resembling Flemming's centres) to squamous acini   Fig 5 shows the transition between the first two, while figs 1 and 2 show low and high power views of the solid acini   None of the epithelial acini show evidence of an invasive tendency , they are bounded by a well defined basement membrane , they do not look malignant and in no way resemble a lympho epithelioma   A typical secondary lympho epithelioma of a cervical lymph node is illustrated in figs 15 and 16 for comparison   The irregularity of its epithelial cell components, absence of basement membrane and invasive tendencies are evident

Case 2   F 67 (*fig 6*)   For years there had been a swelling in the right parotid region but this had recently increased rapidly in size   There were no enlarged lymph nodes and a blood count was normal   The tumour after removal weighed 70 g   It was poorly capsulated   It was of even consistence and there were a few yellow flecks on its streaked and mottled cut surfaces

The histology is similar to that of the first case   Both tubular and solid acinar elements are present and these are set in a lymphoid stroma (fig 6)   The epithelial cells composing the solid acini have not differentiated into cell nests or keratin   At the margins of the tumour there is little capsule formation and there is some lymphoid hyperplasia in the interstitial tissue of the surrounding parotid glandular tissue, as in the first case   The stroma does not appear to be malignant

Case 3   M 29 (*fig 7*)   There had been a history of a swelling in the parotid region for ten years   The tumour was excised and only embedded tissue was received for histological examination

This tumour resembles the first two, particularly with regard to the epithelial elements   There is a tendency for it to be divided into lobules of lymphoid tissue, but these do not appear to be divided from one another by fibrous septa or to be bounded by epithelium , there is no cyst formation   The lymphoid tissue contains the normal anatomical components of a lymph node but the follicles are not very large or numerous   In the medulla, however, there is hyperplasia of reticulum cells and histiocytes

Scattered throughout the tumour are branching tubules and small solid acini (fig 7)   The tubules resemble those of parotid ducts and are surrounded by a thin zone of hyaline fibrous tissue   The solid acini are similar to those in the first two cases, but there is nowhere such well marked squamous differentiation as in case 1 , though they have a well defined basement membrane, they lack an investment of fibrous tissue

The tumour had been damaged by surgical removal so that it is difficult to make out its relationship to the surrounding parotid glandular tissue, but there seems to be a fairly dense zone of fibrous connective tissue between the tumour and the parotid gland

Case 4   F 49 (*fig 8*)   For 8 months there had been a swelling near the right angle of the mandible   This seemed to have appeared after tooth extraction, but an X ray showed no change in the bone and so the swelling was removed   There was also a swelling just below the angle of the left jaw, which was considered to be an enlarged lymph node   No other lymph nodes were enlarged and the spleen was not felt   A blood count only showed a moderate secondary anæmia

The epithelial elements in this tumour are not very well defined but quite often show tubule formation (fig 8)   In other places they form little masses of epithelial cells   The stroma, as in the last case, shows a lympho histiocytic medullary proliferation, which is plainly shown in the illustration

Case 5. F. 31 (figs. 9 and 10). For two years there had been a left pre-auricular swelling. This enlarged rather rapidly at first. It was situated in the substance of the parotid gland near the surface. It was excised in 1942 and has not recurred.

The tumour (2×1·5×1 cm.) was smooth, oval, soft, pink and cellular-looking. It resembled a lymph node. Sections show hyperplasia of the lymphoid stroma with well marked Flemming's centres. Both in these centres and in the medulla are many small branching tubules. Each has a well defined basement membrane of reticulin or sometimes of collagen. There is no tendency to squamous metaplasia or to the formation of solid acini of epithelial cells.

Case 6. F. 60 (fig. 11). For four months there had been a swelling in the right parotid region. There was no pain and the symptoms suggested calculus. On operation, however, none was found and a biopsy was taken of the tumour. This was followed by X-ray therapy, and there has been no recurrence.

The biopsy (1·4×0·9×0·7 cm.) does not show the relation of the tumour to the parotid gland. There is a tendency for it to be divided into lobules by thin fibrous septa. The lymphoid stroma shows follicles, medulla and sinuses and is hyperplastic. It contains numerous branching tubules, evidently derived from the parotid gland, which resemble those seen in case 5.

Case 7. F. 71 (figs. 13 and 14). For about five months there had been a painful swelling in the left side of the neck which had become progressively larger. It was situated in the left submaxillary salivary gland. It was removed and found to be malignant, so that the site was treated with radium needles. Two and a half years later the scar was still healthy and there was no recurrence. During the next year the patient died, probably from some other cause.

The tumour looked like a mass of enlarged lymph nodes (4×3·5×2·5 cm.). The cut surface showed white soft compact tissue with zones of fibrosis. Microscopically the tumour is seen to be divided into lobules by septa of fibrous tissue, which in a few areas is condensed and contains nerve fibres and blood vessels. The lymphoid tissue in this case has been largely replaced by neoplastic cells, which in their appearance and general pattern are exactly like those of a syncytial reticulosarcoma; they have none of the characters of an anaplastic epithelial cell; a reticulin impregnation shows only a few scattered fibres with no particular arrangement, and the tumour cells give a positive silver reaction in the nucleus.

Both in the fibrous tissue and in the zones of lymphoid tissue are tubules and small solid acini of epithelial cells. These have most of the characters of those seen in the preceding cases, but there is no definite squamous metaplasia. The best differentiated tubules resemble the acini and ducts of a salivary gland and are to be found chiefly in the fibrous septa, but there are areas within the lymphoid tissue where these small solid epithelial islands appear to be arising from the tubules by metaplasia. They do not appear to be malignant. They have a well defined basement membrane of reticulin or collagen fibres and their nuclei have a negative silver reaction. Figs. 13 and 14 show some characteristic epithelial islands.

This tumour, then, seems to be an adenolymphoma with syncytial reticulosarcoma of the stroma.

Histological summary

These tumours have the following features in common. They are situated in a salivary gland. They contain glandular elements, tubular, cystic or solid acinar, which are derived from the salivary gland, and which, though in some places they may be only relics of the gland tubules, in most others show evidence of progressive hyperplasia or neoplasia. They have a lymphoid stroma of variable appearance;

Plate CXXXIX

SALIVARY ADENOMA AND ADENOLYMPHOMA
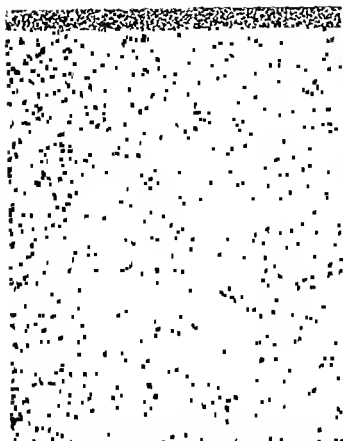
Fig. 9.—Case 5. Reactive hyperplasia of lymphoid stroma. Tubules in follicles and medulla. ×110.

Fig. 10.—Case 5. Reticulin impregnation, showing concentration of reticulin around tubules ×125

Fig. 11.—Case 6. Irregular tubules in a hyperplastic lymphoid stroma ×110
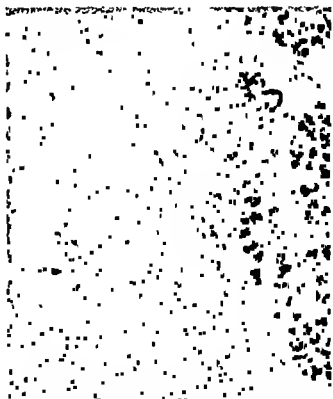
Fig. 12.—Adult parotid lymph node, showing inclusion of glandular acini and tubules ×100.

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Fig. 13.—Case 7. Older looking tubules in a fibrous stroma, with adjacent epithelial island. Completely patternless stroma. ×110.

Fig. 14.—Case 7. Greater magnification to show epithelial island and malignant character of stroma. ×460.

Fig. 15.—Typical secondary lympho-epithelioma of cervical lymph node. ×125.
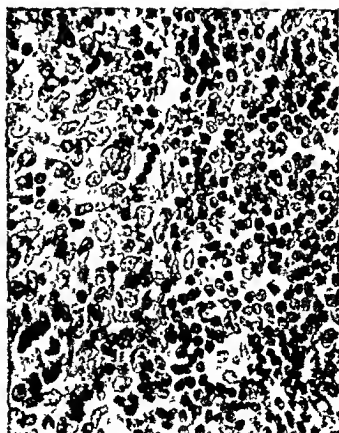
Fig. 16.—Same case as fig. 15, showing the invasive character of the epithelial cells and their irregularity. ×460.

in the first two cases this stroma appeared to arise as a proliferation of lymphocytes in the salivary-gland interstitium and is a simple hyperplasia; in the third and fourth cases the stroma shows a lympho-histiocytic medullary reticulosis, which is a reactive rather than a progressive hyperplasia; in the fifth and sixth cases there is an ordinary hyperplasia of the " reactive " type, with the formation of Flemming centres; while in the seventh there is a syncytial reticulosarcoma.

Although the histological differences are so striking, these cases are so near in their composition to the group of tumours called adenolymphoma of the salivary gland that it seems fair to include them in that group, as Plaut suggests of his three cases. His tumours and that described by Fein are of the same histological type as the seven now described. Plaut mentions cystic spaces hut Fein's case does not seem to have contained cysts. Only case 1 of those now described shows cysts. Škorpil (1939) describes three cases of lympho-epithelioma of the parotid gland, of which the first two are the usual malignant type, but the third looks more like an adenolymphoma of the kind now being discussed, with well defined solid acini of epithelioid cells; moreover, he stated that there was no recurrence after local removal. It is quite likely that other cases have been taken for lympho-epitheliomata.

Origin of adenolymphoma

The similarity of this kind of tumour to those lymph nodes which contain ectopic parotid tubules is too close to escape mention. Neisse (1898) was the first to point out that there were nearly always tubular structures derived from the parotid gland to be found in the pre-auricular lymph nodes of newhorn infants, while Nicholson (1922) developed this point, showing that these glandular structures sometimes persisted into adult life. Plaut says that this work has been repeated and verified in the surgical department at Yale. Fig. 12 shows a pre-auricular lymph node from a male adult in which duct and glandular structures, obviously derived from the neighbouring parotid gland, are to be seen within the lymph node. It is hard to believe, when comparing this with fig. 11, that these tumours are not derived from ectopic glandular epithelium like this.

Is one then to accept the theory that ectopic salivary tissue within a lymph node is the origin of the commoner sorts of adenolymphoma— of those with cysts lined by columnar cells? There are many other theories in this field, of which it seems necessary to mention three. (A more complete account may be found in the writings of Jaffé (1932), Kraissl and Stout (1933), Carmichael, Davie and Stewart (1935) and Bonifazi (1936)).

One theory is that they may be of branchial origin. Bonifazi disposes of this theory by pointing out, in a careful description of

lump occurred in the pre auricular region, and that a portion was sent to the pathologist as a parotid tumour If the section represents a sector of a spheroidal tumour, the original growth might have measured about 3 cm in diameter It is composed of parenchymatous lobules with a minimum of fibrous stroma and with occasional lymphoid aggregates but no germinal centres The cells within these lobules are usually columnar, sometimes spheroidal or polygonal, never ciliated They are arranged in trabeculæ or anastomosing strands, with a suggestion of the palisading described by Hamperl (1937) In such strands there is usually a delicate fibrous connective-tissue basement membrane on both sides and no attempt at tubule formation, elsewhere there is a suggestion of acinus and tubule formation The cytoplasm is granular and distinctly eosinophilic With Masson's trichrome stain it is reddish purple and the granules are prominent The nuclei are neat, round or oval and have a delicate network of nucleochromatin containing usually one but sometimes two or three nucleoli They are usually centrally placed In some parts of the tumour a different type of cell is intercalated This is a flattened almost filamentous columnar cell, which is also sometimes polygonal or star shaped, as though it were compressed by the neighbouring swollen cells Its cytoplasm is homogeneous and hyaline and stains intensely red with basic fuchsin Its nucleus is always pyknotic It appears to be a degenerate form of the principal cell The tumour looks benign, but it is too early yet for the possibility of recurrence to be excluded

On some published cases of salivary adenoma

A pure salivary adenoma, that is one which is neither of the " mixed " type nor an adenolymphoma, seems to be rather a rare tumour Even so there are many kinds, of which the so called oncocytoma is probably the commonest None seems to reproduce the parotid glandular structure Huckel (1933) refers to one in Kaufmann's Lehrbuch (1922, vol ii, p 467, fig 244) This is an idealised drawing of an alleged parotid adenoma, in which there are hollow glandular acini lined by cubical or low-columnar cells and separated by little or no stroma It is impossible from this to recognise what the tumour was There is also the form of adenoma called " para thyroid like ", which is sometimes a variety of the oncocytoma , but intermediate forms occur between the oncocytoma and the adeno lymphoma, the oncocytoma and the mixed parotid tumour and between the adenolymphoma and the mixed parotid tumour

In McFarland's critical study (1927) of all the cases of parotid adenoma which had any claim to be pure, he concludes that they were all variants of the mixed parotid tumour He observes that the adenomas shade off into the mixed parotid tumours and prefers to put them all into the latter group At that time he subscribed to the old theory of Cuneo and Veau, that these " mixed " tumours

the embryology of the parotid gland, that the branchial pouches take no part in its formation. Kraissl and Stout do the same.

Another is that they are derived from the "orbital inclusion", a tubular structure surrounded by mesenchyme, apparently analogous to the parotid gland in its formation from the buccal sulcus but not connected with the gland. Kraissl and Stout describe the formation of this structure in detail and consider that these lympho-epithelial cysts may be derived from it.

The third is that of Hamperl (1931), which must be treated in rather more detail, not because it is more probable but because of its bearing on the formation of the pure adenoma. Hamperl examined histologically the salivary glands of a large number of cases coming to post-mortem and he describes swollen non-ciliated columnar or cubical epithelial cells with an intensely eosinophilic granular cytoplasm, which he calls "onkocytes", because of their swollen appearance. These were absent from the parotid glands of persons under 20 years of age but were present to an increasing extent in those of riper years, appearing in every case over 70. Sometimes they occurred singly, sometimes in groups, sometimes forming little hyperplastic nodules, sometimes forming true neoplasms. Hamperl assumed that they were derived from the glandular parenchyma by a process of dedifferentiation; their nuclei were usually pyknotic (hence the older name of "pyknocyte"), but this did not appear to interfere with their reproductive potential. He also pointed out that oncocytes occur in all sorts of mixed and mucous glands of the tongue, throat and alimentary and respiratory channels. Stout (1943) has more recently drawn attention to similar cells in the bronchi and has suggested that one type of bronchial adenoma may be derived from them.

Hamperl maintains that all adenolymphomata are derived from these oncocytes. Jaffé enlarges on this hypothesis and suggests that the name "oncocytoma" be substituted for "adenolymphoma". Ackerman (1943), on the other hand, thinks that the name "oncocytoma" should be used only for that type of salivary adenoma which appears to consist purely of oncocytes and has no lymphoid stroma. The latter seems much too close a restriction, considering the wide distribution within the body claimed for the oncocyte. Similar tumours also occur in the palate (Ahlbom, 1935) and under the conjunctiva; the author has encountered a case in the lachrymal caruncle of a woman of 76.

A case of oncocytoma of the parotid

The tumour here described seems to be a parotid adenoma corresponding closely to the type called oncocytoma, but containing a few small lymphoid aggregates.

Case 8. F. 59 (fig. 17). No history is available, except that the

PLATE CXLI

SALIVARY ADENOMA AND ADENOLYMPHOMA

se 9. Parotid adenoma, showing
ules and trabeculæ of cubical
25.

FIG 19 —Typical adenolymphoma; the epithelium resembles that of parotid tubules, both morphologically and in being two cells thick. ×55.

FIG 20.—Typical adenolymphoma. the basal layers of the epithelium multiplied. ×90.

grew from residual embryonal vestiges of ectodermal, mesodermal and hypodermal structures. In 1942, however, he writes of "the so-called 'mixed tumours'", which suggests that he is no longer satisfied with the old theory. It would probably be more in accordance with modern tendencies, as set forth for example by Harvey, Dawson and Innes (1938), to classify the mixed parotid tumours with the adenomas.

Ackerman gives the latest tabulation of recorded cases of the oncocyte group of salivary adenoma; he enumerates eight. Huckel (1930) describes two cases of parotid adenoma which have been quoted as being parathyroid-like. One seems to have been an oncocytoma, the other was probably a "mixed" tumour. Franssen (1932-33) describes an adenoma of the parotid which he says is like Hückel's cases and he calls it parathyroid-like. The cells were polygonal, sometimes arranged in trabeculæ and had hæmatoxyphilic cytoplasm with water-clear vacuolation. These vacuoles did not contain fat or mucus but the material was not stained for glycogen. He describes the nuclei as rather large, round and containing one nucleolus and rather a lot of chromatin. In his illustration, however, the nuclei are small and dark. There seems no good reason for not including this tumour among the oncocytomas. Hückel (1933) followed this up with another "parathyroid-like" tumour of the parotid, in which there was a tendency to tubule-formation. From his description (there is no illustration) the tumour would fit quite well into the "mixed" group.

Ahlbom illustrates two cases of adenoma (plates 29 and 30), one of the parotid, the other of the hard palate. The latter is tabulated in Ackerman's list of oncocytomas; the former might well have been included also but the cytology was spoilt by pre-operative irradiation. The cells show much water-clear vacuolation. Both were radiosensitive.

Leroux and Leroux-Robert (1934) in their histological classification of salivary-gland tumours illustrate, in their second figure, a palisaded adenoma not unlike my case 8. In their figs. 15 and 16 they illustrate a tumour resembling the so-called parathyroid-like variety, which they describe as a "forme acineuse pure à cellules claires". The cells do not really form true acini but are grouped around capillaries and have vacuolated cytoplasm and a "disposition pseudo-endocrinienne". Here the resemblance to Hückel's and Franssen's cases ends, for they go on to say that these tumours (of which they have encountered three cases) both look malignant and behave in a malignant fashion, recurring repeatedly and in one case giving rise to pulmonary metastases.

Thus the "parathyroid-like" group of salivary tumours would seem to include some oncocytomas, some "mixed" and some malignant types. There really seems no reason to distinguish them by a separate name.

PLATE CXLI

SALIVARY ADENOMA AND ADENOLYMPHOMA

FIG 17.—Case 8 Adenoma of the "oncocytoma" kind Trabeculæ of cubical or columnar cells with eosinophilic cytoplasm A small lymphoid aggregate is also present ×125

FIG 18 —Case 9 Parotid adenoma, showing cysts, tubules and trabeculæ of cubical cells ×125

FIG 19 —Typical adenolymphoma, the epithelium resembles that of parotid tubules, both morphologically and in being two cells thick ×55

FIG 20 —Typical adenolymphoma, the basal layers of the epithelium multiplied ×90

Another case of parotid adenoma

This tumour seems to occupy a place midway between the cystic adenolymphoma and the mixed tumour. Though it has not yet recurred clinically it shows certain malignant histological features (but see McFarland, 1942, for the value of these).

Case 9. *F.* 69 (*fig.* 18). For 6 to 8 months there was an unsightly painless lump growing in the right parotid gland. The patient was otherwise well, except for a hypertension of 300 mm. Hg. systolic. The tumour was removed on 27th March 1944 and there was no recurrence by October 1944.

When removed the tumour appeared to be partly cystic and most of the fluid contents escaped. Otherwise it was a firm, partly rounded, partly irregular mass, $3 \times 2 \times 1 \cdot 5$ cm., with a tough fibrous capsule covering the rounded part. The cut surface showed slight lobulation by fibrous tissue septa of a soft haemorrhagic growth.

Microscopically capsule and septa are seen to consist of old collagenous fibrous tissue containing a few foci of chronic inflammatory cells with siderotic histiocytes, many areas of lymphoid tissue and a few places where the tumour has invaded the capsule. There is no parotid glandular tissue but at one edge are some quiet-looking, sometimes dilated gland tubules lined by flattened, cubical or columnar epithelium. The parenchyma consists of cysts, tubules and trabeculæ of cubical or low-columnar cells. Owing to the paucity of stroma within the tumour mass the cyst walls often look like undulating strands uniformly two cells thick. One of the more solid areas is depicted in fig. 18. The cysts and tubules sometimes contain much mucoid material, sometimes extravasated blood. The tumour cells have each a round centrally placed nucleus with well marked chromatin network and from one to three nucleoli. Mitoses are few. Giant nuclei occur and are hyperchromatic or sometimes vacuolated. The cytoplasm is rather basophilic, granular and sometimes vacuolated. The details which suggest that the tumour might be malignant are the irregularity in character and arrangement of the cells and the invasion of the capsule.

On the origin of salivary adenomata

Though it was not originally intended to discuss the ontogenesis of the mixed salivary tumours, it is necessary to do so because of the types of adenoma, which from a histological point of view occupy a position intermediate between the pure adenoma and the mixed type.

A useful historical survey has been made by Ahlbom in one of his introductory chapters to a volume containing the results of radiological treatment of 254 tumours of mucous- or salivary-gland tissue. In this he points out that, with the exception of the earliest and least well grounded views, the original theory that they were adenomata derived from salivary-gland tissue was developed in France about the middle of the 19th century and has more or less held the field there ever since. In the German-speaking countries, however, it was the idea first of a mesenchymal and then of an endothelial origin that held sway, both eventually giving place to one or more of the developments of Cohnheim's theory—that the mixed tumours were truly "mixed" in the sense that they were derived from embryonal or branchial anlagen capable of developing epithelial, mesenchymal

and glandular structures. More recently a return has been made to a modification of the old French idea, that these tumours, if not actually derived from salivary-gland tissue, are at least always epithelial in origin. Even so, some doubt exists whether the myxomatous and cartilage-like structures found are derived from epithelium or mesenchyme. Hemplemann and Womack (1942) claim to be able to distinguish histochemically between the mucins of epithelial and of connective-tissue origin and state that they find both in salivary-gland tumours, while Harvey *et al.* maintain that none of the " cartilage " is true cartilage and that both this and the myxomatous tissue is formed by the fibrous connective tissue imbibition of the mucoid products of auto-destruction of the mucin-secreting parenchyma. They admit, however, that the myxomatous tissue may sometimes be the product of autogenous metamorphosis of the fibrous connective tissue stroma, and they draw attention (as does Ahlbom) to the common appearance in the mammæ of bitches of tumours identical in appearance with mixed salivary tumours, which may even develop in their stroma true cartilage or bone.* They conclude that the mixed tumour of salivary glands arises from embryonal glandular replacement epithelium, which may or may not be sequestrated.

With this conclusion one must agree and it makes the starting point for a contention that all salivary adenomata, whether mixed or pure, have a similar origin. There seems no reason to doubt that the oncocytoma is derived from the cells described by Hamperl (" onkocytes ") by a neoplastic process, but in accordance with this contention the oncocyte is not a product of dedifferentiation of the salivary epithelium but arises by the one-sided differentiation of embryonal glandular replacement epithelium. The fact that oncocytes are not found in the young is no contradiction, since it is explicable on the ground that the differentiation assumed does not take place until later in life. When the differentiation is less one-sided, those tumours may be produced which have histological characters intermediate between the oncocytoma and the mixed salivary-gland tumour. .

The adenolymphomas seem at first to occupy a rather special position on account of the lymphoid nature of their stroma. Hamperl would like to include them with his oncocytoma group, and indeed it is possible to find oncocytomas with a greater or lesser development of lymphoid tissue in their stroma. But the tall columnar epithelium found in adenolymphomas, together with their papillary cyst formation, seems to put them in a different category. In their tendency to squamous metaplasia, however, they show a characteristic paralleled by the mixed type of tumour.

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* The role of the myo-epithelium in the formation of the stroma of these tumours has been convincingly demonstrated by Sheldon (1943).

Lymphoid hyperplasia in association with glandular or opithelial hyperplasia of inflammatory or endocrine origin is a common phenomenon in the mouth, pharynx and gastro-intestinal tract, and in the structures derived from the branchial clefts, of which the thyroid and thymus are outstanding examples. Lymphoid hyperplasia in association with neoplastic processes is less common, but is characteristic of lympho-epitheliomata of the mouth and pharynx (figs. 15 and 16) and of the thyoma, and it is also seen in the seminoma and dysgerminoma.

It would seem reasonable, therefore, to regard the adenolymphoma as being a benign tumour derived from the same embryonal glandular replacement epithelium as that which gives rise to the other salivary adenomas, but as differing from them chiefly in having a greater amount of associated lymphoid stroma. Those from the parotid region may well be derived in some cases from the orbital inclusion described by Kraissl and Stout, which may be assumed to contain some of these embryonal glandular replacement epithelial cells. Reasons have already been given for regarding most of the seven cases described in this paper as being sufficiently closely related to the adenolymphoma to be included in that group, though it might be useful to distinguish them by some such epithet as " solid ". Stress is sometimes laid on the fact that the histology of the adenolymphoma is so characteristic, and so in many cases it is ; but since intermediate forms occur between the typical adenolymphoma and the pure adenoma or the oncocytoma, it seems unnecessary to postulate a different origin for the former. The associated lymphoid hyperplasia is not very remarkable when one considers the intimate relationship which often exists between salivary glandular tissue and the local lymph nodes.

Summary

Seven cases of a solid variety of adenolymphoma are described. Six were benign, while the seventh showed a syncytial reticulosarcoma of the stroma. Of the benign cases five occurred in women and one in a man, chiefly in the 3rd, 4th, 5th and 6th decades. They were painless and often had ill-defined outlines. They only recurred if incompletely removed and seemed to be radiosensitive.

Reasons are given for classing them with the adenolymphomas. They should not be confused with the lympho-epithelioma, which is a carcinoma. Four or five cases have been found in the literature of which the correct nature of three was recognised. It is probable that many other cases lie concealed amongst the lympho-epitheliomata which have been described as benign.

A survey has been made of the literature of the salivary tumour known as " oncocytoma " and a case is described. Reasons are given for considering that all forms of salivary adenoma are closely related and that intermediate forms between all types can occur. A form

intermediate between the adenolymphoma and the mixed salivary gland tumour is described.

Consideration has been given to the origin of salivary adenomas and it is concluded that they all arise by the one-sided differentiation of embryonal glandular replacement epithelium. The presence of the lymphoid stroma of the adenolymphoma is explained by the close association of lymphoid and epithelial tissues in the salivary glands and elsewhere in the mouth and in the pharynx and its derivatives.

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HÆMOLYTIC ICTERUS (ACHOLURIC JAUNDICE), CONGENITAL AND ACQUIRED *

J. F. LOUTIT and P. L. MOLLISON

From the South London Blood Supply Depot

TO-DAY, in spite of notable advances in hæmatology in the last two decades, the hæmolytic anæmias are little better understood than in the years preceding 1914, when such well known Continental figures as Minkowski, Landsteiner, Vaquez, Chauffard, Widal and Banti and their pupils studied them. Modern classifications agree on four known ætiological factors : (1) Congenital defects of the erythron, *e.g.* sickle-cell anæmia, (2) bloodstream infections (streptococcal, staphylococcal and *welchii* septicæmias etc., and malaria), (3) exogenous toxins (lead, phenylhydrazine, arsine, toluylenediamine etc.), and (4) endogenous antibodies leading to red-cell destruction, (*a*) the cold iso-hæmolysins in paroxysmal hæmoglobinuria, (*b*) the natural blood-group iso-antibodies in incompatible transfusions and (*c*) the immune maternal iso-antibodies causing hæmolytic disease of the fœtus and newborn. Many cases of anæmia, clinically hæmolytic in character, cannot with certainty be attributed to any of these causes.

Probably the commonest type of hæmolytic anæmia seen in this and other temperate countries is that known as hæmolytic icterus or acholuric jaundice. Both these designations and the ordinarily used synonyms are non-specific terms and do not serve to identify the syndrome from other hæmolytic states. Even when prefixed with the adjective " familial " they do not distinguish it from other hæmolytic states which are familial, *e.g.* sickle-cell anæmia and elliptocytosis (Penfold and Lipscomb, 1943). A congenital and frequently familial form has been generally accepted : an acquired form has been described but not generally accepted. Both types have certain clinical and hæmatological features in common. The subjects are usually clinically jaundiced and have hyperbilirubinæmia, but no bilirubin in the urine ; they have an enlarged and usually palpable spleen and usually present an anæmia of a degree ranging from mild to severe ; the blood shows an increased reticulocyte count, spherocytosis of the non-reticulated cells and increased fragility of the red cells to hypotonic saline. The hæmolytic basis of the

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syndrome is proved not only by the signs of excessive red-cell regeneration in the peripheral blood, but also by the constantly excessive bilirubinoid pigment excretion in the urine and fæces and by the constantly increased rate of autohæmolysis of the blood *in vitro*.

The first descriptions of the congenital form are fragmentary. Murchison (1885) described a family of which several members were jaundiced. Wilson (1890) noted in another such family chronic splenic enlargement in individual cases. Minkowski (1900) gave the first reasonably complete description. He found 8 cases in three generations of a family and noted the chronic icterus, urobilinuria and splenomegaly and at post-mortem on one case, hæmosiderosis of the organs. He attributed the condition to an abnormality of blood destruction due to a lesion of the spleen. Chauffard (1907) found the same clinical features and first recorded the increased fragility of the red cells to hypotonic salines. With Fiessinger (Chauffard and Fiessinger, 1907) he also described the vital staining of the red cells and the increase of " granular " cells or reticulocytes, different from the stippled red cells of plumbism. Microcythæmia as a feature of the blood films has been mentioned from the earliest times. Naegeli (1931) showed that these microcytes were spheroidal cells and coined the term " globe-cell " anæmia.

The first report of the acquired form is generally attributed to Hayem (1898), but it was Widal, Abrami and Brulé (1907-12), who in a series of admirably lucid papers, clearly distinguished the features of the acquired from the congenital form. According to them, in contrast with the congenital case who, Chauffard had noted, was "icterique plutôt que vraiment malade", the acquired cases were severely ill and anæmic ; there was no past or family history of the condition ; they were all adults ; their red cells were usually not the microcytes of the congenital form, although anisocytosis was marked and signs of regeneration were as evident as in the congenital cases ; the fragility of the red cells was increased, especially if the red cells were washed free of plasma before being suspended in the various saline solutions : auto-agglutination occurred ; spontaneous and complete recovery was possible ; all the features could be reproduced in dogs by intraperitoneal injection of toluylenediamine.

In spite of this clear differentiation, in the last two decades there has been a tendency to regard both clinical forms of the syndrome as of identical ætiology. This view was held by Tileston (1922) in the U.S.A., and Paschkis (1930) in Europe. Dawson (1931) and Vaughan (1936) in this country were of the opinion that all were cases of the congenital form. Dacie (1943) regarded a chronic course and a positive family history as confirmatory only and not essential for the diagnosis of " familial hæmolytic anæmia ". Both Dawson and Paschkis have explained the apparently acquired cases as being latent until the syndrome manifested itself, and in some of their cases at least this was the correct explanation. Both concluded that, if a

sufficiently large number of relatives were subjected to the saline osmotic fragility test, other latent cases with increased red-cell fragility would be found in the family. Meulengracht (1922), however, has stated that the condition is not passed on by cases of the acquired form to their offspring. On the other hand, Dameshek and Schwartz (1940), although they recognised the clinical difference between the congenital and acquired forms, seemed to regard them as being of probably the same ætiology and due to a " hæmolysin ".

Evidence is presented below by a study of the survival time of transfused red cells and of the sensitisation of red cells that the congenital and the acquired cases show physiological and serological differences from each other, indicating that they have a different ætiology.

METHODS

Estimation of survival of transfused red cells. Dacie and Mollison's (1943) or Mollison and Young's (1940–41) modification of the Ashby (1919) technique was used for the detection of heterologous but compatible red cells in a recipient's circulation, e g group O red cells in a group A recipient, or type Rh− red cells in a type Rh+ recipient, or type N red cells in a type M or MN recipient. A count of the unagglutinated red cells in a blood sample taken within a few minutes of the end of a transfusion was regarded as 100 per cent. survival. All subsequent counts of unagglutinated cells were expressed as a percentage of this figure and, as pointed out by Mollison and Young (1941–42), this may result in a slight overestimate of the true survival.

Freshly drawn blood or blood stored for a few days only in a reliable glucose citrate preservative was used for the transfusions.

All the cases investigated manifested the diagnostic criteria given above, including the spherocytosis and increased red-cell fragility.

Estimation of sensitisation of red cells. A 2 per cent. (approx.) suspension in normal saline of thrice washed red cells from individual patients was mixed with equal quantities of a rabbit anti human serum serum, which had been suitably absorbed with human A, B and O cells. The mixture was allowed to stand at room temperature for one hour and then inspected for agglutination as described by Coombs, Mourant and Race (1945). The presence of agglutinates was taken to indicate that the red cells had adsorbed an immune antibody-globulin from the plasma, thus sensitising them, and that the adsorbed globulin, remaining adsorbed during the washing process, had interacted with the anti-human serum serum to cause agglutination of the red cells. The agglutination was recorded as +++ (complete or visual agglutination), ++ (strong agglutination when viewed microscopically) or + (moderate agglutination when viewed microscopically). As different batches of anti human serum serum were in use and as no standardisation had been carried out, the results are considered of qualitative value only.

RESULTS

Transfusion of normal blood to recipients with acholuric jaundice

(a) *Congenital and familial type.* Dacie and Mollison have already published their results of the transfusion of normal blood to recipients with congenital acholuric jaundice. Fig. 1 shows the survival of the normal red cells in 5 such cases. The figure is copied from Dacie and

Mollison, who published 6 cases, but the sixth case has been omitted; it was considered atypical in that the patient was an Rh— recipient who developed an anti-Rh agglutinin. It was felt that this phenomenon accounted for the diminished survival of the Rh+ transfused red cells in this case. Normal blood in these five cases survived for periods of 100-120 days and the graph of elimination was roughly linear. These findings are such as are obtained in normal recipients (Mollison and Young, 1941-42; Brown *et al.*, 1944; Callender *et al.*, 1945).
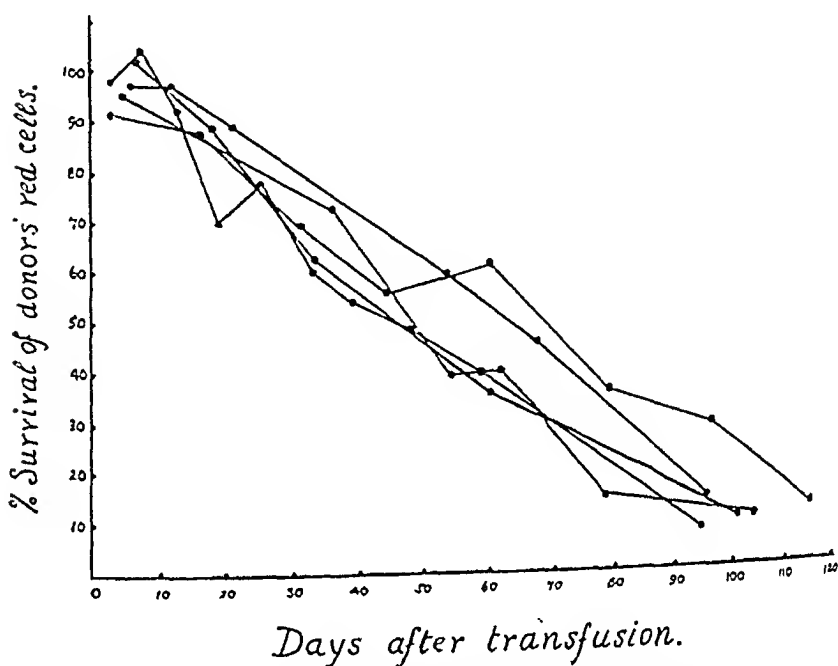
Fig. 1.—Survival of transfused normal red cells in 5 recipients with congenital acholuric jaundice. (Data derived from Dacie and Mollison, 1943).

(b) *Acquired type.* In 8 such cases transfused with normal blood the results were totally different. Cases 1-4 reported briefly here are from an as-yet-unpublished series of cases of hæmolytic anæmia of various types which have been studied in some detail (Mollison).

Case 1 (E. S., ♀ aged 44) received, before splenectomy, at an interval of 6 months two transfusions, the survival of the red cells of which was followed. Both showed very rapid destruction of the transfused red cells, 50 per cent. having been eliminated in 5 and 3 days respectively.

Case 2 (W. E., ♂ aged 65). Here the rate of elimination was still more rapid.

It is notable that in both cases the survival time estimated a few days after splenectomy, though still much less than normal (50 per cent. survival at 9 and 4 days respectively), showed a definite improvement when compared with the figures before operation. It is even more notable that in case 2 the survival time 2 years later was still greatly diminished (50 per cent. survival at 8 days), although this man, now aged 67, was doing a full seven-day week's work and

cauld " walk far miles and miles ". He died suddenly $2\frac{1}{2}$ years after splenectomy from pulmonary embolism following thrombosis of the crural veins. Case 1 is alive and well $3\frac{1}{2}$ years after splenectomy. In both cases spherocytosis and increased red cell fragility persisted after the operation.

Case 3 (M. C., ♀ aged 56) in 1943 presented all the phenomena of acquired acholuric jaundice; she also eliminated transfused red cells very rapidly (50 per cent. survival at 5 days). She refused splenectomy, but, like one of the cases reported by Widal *et al.* (1909), subsequently made a spontaneous and apparently complete clinical recovery. The blood count became normal and in contradistinction to the two previous cases the increased red cell fragility and spherocytosis disappeared. A further test blood transfusion in 1945, 2 years later, showed normal survival of the transfused red cells as far as they were followed for 3 weeks, a striking contrast with the previous cases.

♀ æt 28
Splenectomy 4 × 45

% Survival of donors' red cells

Days after transfusion
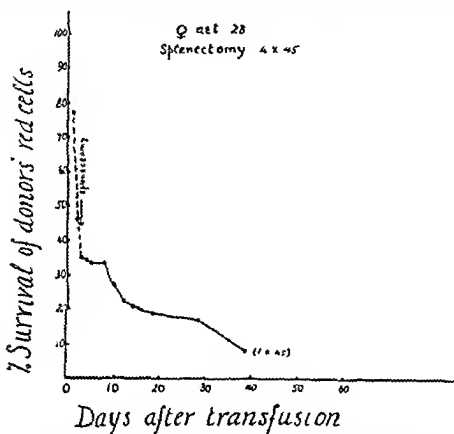
FIG 2.—Survival of transfused normal red cells in case 5 of acquired acholuric jaundice. Dotted line, survival graph before remission Continuous line, survival graph after remission, following splenectomy

Case 4 (M. S., ♀ aged 60) also showed all the features of acquired acholuric jaundice. She received several transfusions in the course of a few weeks. In each instance 50 per cent. survival was charted at 7 days or less. She died without splenectomy having been carried out.

In addition to these earlier cases, 4 other cases (nos. 5-8) have been investigated.

Case 5 (R. L., ♀ aged 28) was a typical example of the acquired acholuric jaundice syndrome. As splenectomy in cases 1 and 2 had seemed to produce not only clinical improvement but also an apparent slight lengthening of the survival time of transfused red cells, it was decided in this instance to follow the survival of red cells transfused 3 days before the splenectomy. Red cells from the same blood could thus be traced both before and after the operation. The rate of elimination, very rapid indeed before splenectomy (two-thirds approximately of the transfused red cells had already been destroyed), was obviously markedly slowed following the operation (fig. 2), though it was still

greater than normal. A detectable residuum was present 40 days after the transfusion. Six months after the operation her blood count, like that of cases 1 and 2, was within normal limits; like cases 1 and 2 also, the spherocytosis and increased red cell fragility persisted as in cases of the congenital type.

Case 6 (E. P., ♀ aged 44) was clinically and hæmatologically similar to the previous cases. However, splenectomy appeared to have no effect on the rate of elimination of transfused red cells, which was equally rapid (about 50 per cent. survival at 2 days in both instances: fig. 3) when transfusions were given one month before and 6 days after operation. Clinically the splenectomy seemed to have caused some remission of the hæmolytic state, but there was persistent pyrexia and finally an obvious relapse with increased hæmolysis associated with widespread intravascular thromboses which resulted in her death.
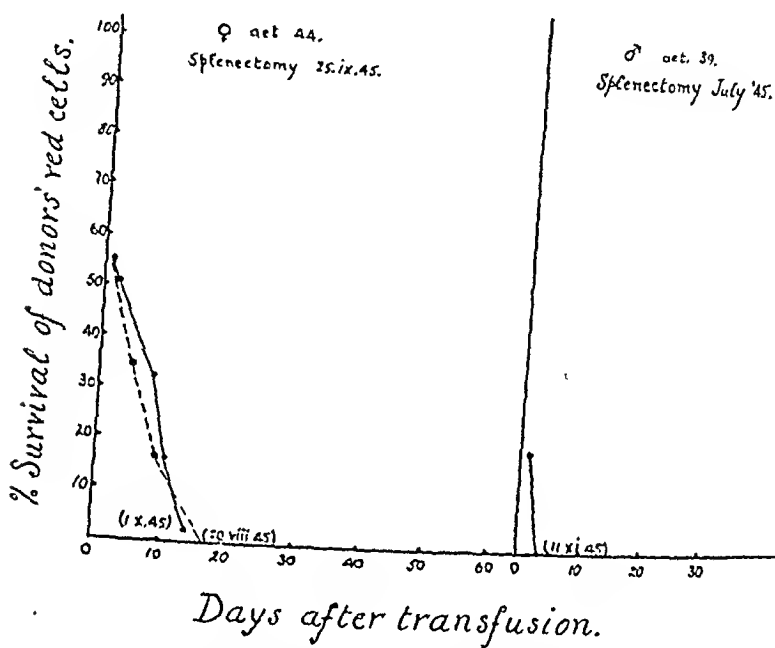
FIG. 3.—Survival of transfused normal red cells in cases 6 and 7 of acquired acholuric jaundice. Dotted line, transfusion before splenectomy. Continuous lines, transfusions after splenectomy.

Case 7 (J. W., ♂ aged 39) had bilateral fibro-caseous pulmonary tuberculosis and apparently developed the syndrome of acquired acholuric jaundice in the spring of 1945. He might thus be regarded as a case of secondary or symptomatic acholuric jaundice, although there is no generally recognised association of the syndrome with tuberculosis. He presented all the features of the syndrome described above. Splenectomy in July 1945 produced no lasting improvement. He was first seen by us in the autumn of 1945. Elimination of transfused red cells at this time was extremely rapid (20 per cent. survival after 24 hours: fig. 3). He died shortly afterwards.

Fig. 4 refers to a single case (G. B., ♂ aged 39) with no family history but a past history of periodic jaundice extending back for about 20 years. He was first investigated in 1943, when he had infective mononucleosis and a relapse of his jaundice. The survival test of the transfused blood was made after recovery from the infective mononucleosis and when he was, as regards his anæmia and jaundice, in a state of remission. It will be noted that his elimina-

tion graph is curved and that 60 per cent. of the transfused red cells had been destroyed within 32 days. The diagnosis in this case between congenital and acquired acholuric jaundice was somewhat equivocal, but there was a complete absence of family history, and his mother, his only surviving relative, had a normal red-cell fragility. He was subsequently submitted to splenectomy. The blood count returned to normal figures, but the red cells remained unduly fragile to hypotonic saline. No transfusions were performed after the operation. He died unexpectedly 18 months later from a coronary thrombosis. This case has been included, not as a definite case of acquired acholuric jaundice, but for completeness.
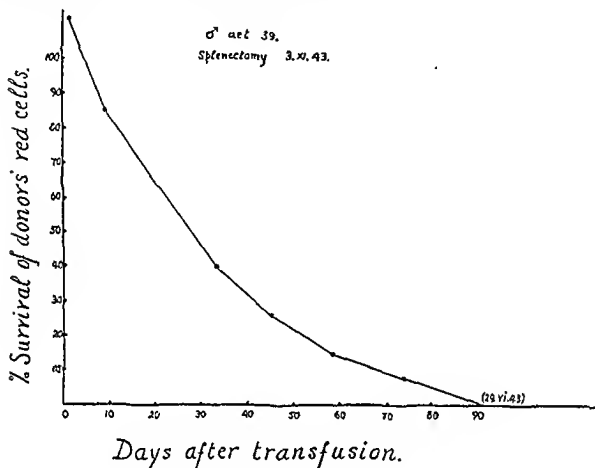
Fio. 4.—Survival of transfused normal red cells in case 8 of acquired acholuric jaundice before splenectomy.

To sum up, eight cases with the clinical diagnosis of acquired acholuric jaundice have shown an increase in the rate of elimination of transfused red cells—very marked in seven cases, slight in one. In some of the cases the graph of elimination was not, as in the normal recipient, linear, but curved and of roughly exponential form (*cf.* Brown *et al.*). Any variations from the perfect curve may have been due to an error in the method or, in some cases, to variations in the activity of the hæmolytic process.

Transfusion of blood from patients with acholuric jaundice to normal recipients

The recipients of these transfusions were all patients with simple post-hæmorrhagic anæmia in whom the survival of transfused blood

has been shown to be similar to that in the normal recipient (Brown et al., 1944; Callender et al., 1945; Mollison, in preparation, 1946).

(a) *Blood from cases of congenital and familial type.* Blood was drawn from 3 such patients, 2 of whom had intact spleens; the third had had a splenectomy one year previously and the results of this transfusion were noted by Dacie and Mollison. The red cells of these 3 bloods, after transfusion into recipients with post-hæmorrhagic anæmia, were eliminated far more rapidly than normal red cells (fig. 5), in spite of the fact that in the 2 unsplenectomised cases at least the reticulocyte count was high and therefore the average age
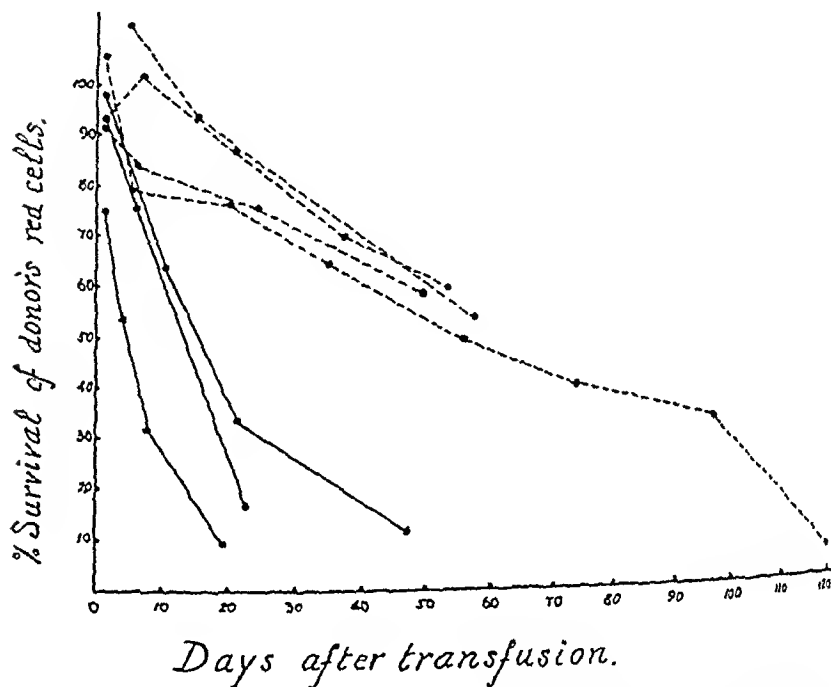
FIG. 5.—Survival in normal recipients of transfused red cells from 3 cases of congenital acholuric jaundice (continuous lines), and 4 cases of acquired acholuric jaundice (dotted lines).

of the individual donor red cells should have been less than in a normal blood. Had the cells been normal cells one might have expected a maximal life-span of 100-130 days, but with an average cell life greater than normal; that is, the graph would not have been linear but curved with the convexity uppermost. In fact the graphs tend to be concave rather than convex and a 50 per cent. survival is indicated at about 5-15 days instead of the normal 50-60 days.

(b) *Blood from cases of the acquired type.* Blood was drawn from 4 such patients—in cases 2 and 5, 22 months and 1 month respectively after splenectomy; in case 4 after her spontaneous and apparently complete clinical recovery; in case 8 before splenectomy. *In cases 2, 5*

and 8 the patients were in a state of clinical remission, but there were still signs of active disease such as spherocytosis, increased fragility to hypotonic saline and rapid elimination of transfused normal red cells. The red cells from these patients survived in the recipients with post-hæmorrhagic anæmia in a manner indistinguishable from normal red cells (fig. 5). In two instances (the blood from cases 2 and 5) the survival figures one week after transfusion were 84 and 79 per cent. respectively, that is rather less than the expected figure of 90-100 per cent. for normal red cells, but the rest of the graph in each case suggests a normal survival. Although, as has been pointed out, the diagnosis was equivocal in case 8, the results of the transfusion of his blood have been included, as they differ from those obtained with the blood of cases of congenital acholuric jaundice and are similar to those obtained with blood of cases of the acquired type.

Serological experiments

In a preliminary communication (Boorman, Dodd and Loutit, 1946) the results have been reported of the agglutination reactions of the washed red cells from cases of acholuric jaundice with anti-human-serum serum. Table I, copied from this report, shows that

TABLE I

Results of the agglutination test with anti-human-serum serum and the washed red cells of 17 cases of congenital acholuric jaundice

Age	Sex	Hb (per cent)	Plasma bilirubin (mg. per cent.)	Reticulocytes (per cent)	Splenectomy	Agglutination of red cells 8. anti-human-serum serum
10	♂	50	19·2	10·0	—	—
...	♂	53	3·2	9·5	—	—
10	♂	92	4·8	12·0	—	—
23	♀	...	3·0	11·0	—	—
...	♂	85	2·6	...	—	—
18	♀	80	2·7	12·0	—	—
50	♀	76	5·7	7·0	—	—
...	♀	98	0·16	0·5	—	—
...	♂	...	0·32	0	+	—
69	♀	107	0·7	1·5	+	—
5	♀	86	0·5	3·0	+	—
40	♀	96	0·8	0·5	+	—
12	♀	90	0·7	0	+	—
18	♀	90	0·5	0·3	+	—
78	♀	82	2·7	4·0	+	—
30	♀	85	1·6	1·0	+	—
24	♂	...	...	...	+	—

in 17 cases of congenital acholuric jaundice the red cells were not agglutinated by the anti-human-serum serum : 16 were classical cases of the disease, the 17th was the mother of a child with the classical syndrome, who herself showed no stigmata except increased red cell

fragility. Of the other 16, 9 had had splenectomy and the 7 with intact spleens were moderately or slightly anæmic.

Table II shows the results of the agglutination tests in 7 cases of acquired acholuric jaundice. In 3 of these the test was carried out

TABLE II

Results of the agglutination test with anti-human-serum serum and the washed red cells of 7 cases of acquired acholuric jaundice

Case no.	Age	Sex	Date tested	Hb (per cent.)	Plasma bilirubin (mg. per cent.)	Reticulo-cytes (per cent.)	Date of splenectomy	Agglutination of red cells c̄. anti-human-serum serum
2	67	♂	27.x.45 (Post-mortem blood)	...	...	...	21.iv.43	+
1	46	♀	20.x.45	98	0·16	1·0	1.i.43	++
6	44	♀	13.ix.45	47	7·2	51·0	26.ix.45	+++
			19.ix.45	62	4·8	8·0		+++
			29.ix.45	72	1·3	6·0		++
			1.x.45	70	3·2	5·0		
			12.x.45	61	3·6	3·0		
7	39	♂	20.ix.45	38	...	28·0	July 1945	+++
			17.x.45	38	4·8	31·0		++
3	29	♀	27.ix.45	30	3·3	...	4.x.45	++
			1.x.45	39	...	52·0		++
			8.x.45	100	...	1·0		+
			12.x.45	78	...	1·0		+++
			20.x.45	81	0·9	0·5		+++
			9.xi.45	86	...	5·0		++
4	59	♀	7.ii.46	102	0·3	0·7	Spleen intact	++
9	74	♂	11.ii.46	52	8·0	52·0		++

with the patient in a stage of relapse and with an intact spleen. In 3 cases the test was only done after splenectomy and in one (case 4) when the patient had made an apparently complete clinical and hæmatological recovery. In all instances the agglutination test was positive.

These results suggest that the red cells of cases of acquired acholuric jaundice have adsorbed from the plasma an immune antibody, whereas the red cells of cases of congenital acholuric jaundice have no adsorbed immune antibody.

Additional experiment

The following experiment is one of an unpublished series (Boorman, Dodd, Loutit and Mollison). Persons of group A were bled 400 c.c. into citrate solution. The blood was immediately centrifuged, the supernatant plasma removed and the red cells re-suspended in an

anti-A serum of high titre. In individual experiments the amount of anti-A serum added was varied. This resulted in varying degrees of clumping (apparently agglutination) of the red cells. The maximum amount of anti-A serum added was 180 c.c. to 220 c.c. of packed A red cells. These mixtures were allowed to stand for about an hour at room temperature and were then transfused to recipients with post-hæmorrhagic anæmia, in whom the survival of the clumped and presumably sensitised red cells could be followed. Fig. 6 shows that in all 5 cases so transfused the survival of the sensitised blood was apparently normal for as long as it was followed.
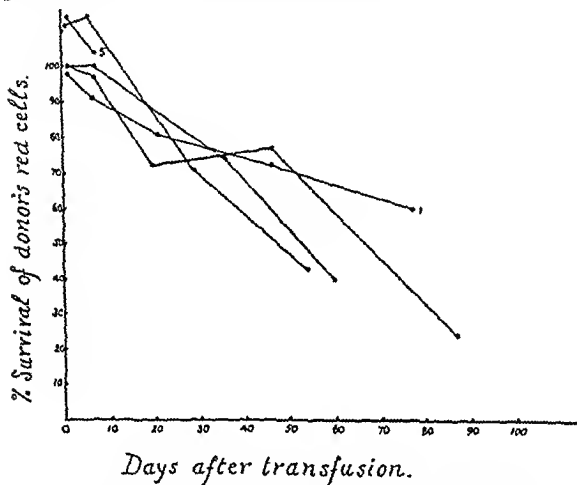
FIG. 6.—Survival in normal recipients of group A red cells sensitised with anti-A serum.

DISCUSSION

There are several theories of the ætiology of acholuric jaundice, most of which have been formulated on the basis of there being merely one syndrome of acholuric jaundice and not two.

Thus Naegeli, who first described the spherocyte, suggested that it was pathognomonic of congenital acholuric jaundice and represented an inborn developmental defect of the red cell. Other workers (Gänsslen, 1922; Haden, 1934; Thompson, 1936; Vaughan, 1936; Gripwall, 1938; Lloyd, 1941) have agreed with this view. On the basis of this hypothesis acholuric jaundice of the congenital variety might well be called globe cell anæmia as suggested by Naegeli, as it would be comparable with sickle-cell anæmia and elliptocytosis.

On the other hand, Dameshek and Schwartz (1938b), who produced

acute and subacute hæmolytic anæmias with spherocytosis in animals by means of an immune hæmolytic serum, believe that spherocytosis is due to the action of the hæmolysin *in vivo* on mature red cells and they (1940) rightly point out that spherocytosis is not pathognomonic of congenital acholuric jaundice. Spherocytosis has been recorded, or has been inferred from increased red-cell fragility, in acute hæmolytic anæmia (Dameshek and Schwartz, 1938*a*), myelosclerosis and allied syndromes (Vaughan and Harrison, 1939; Weil and Perlès, 1938), leukæmia (Heilmeyer, 1936; Singer, 1940), erythroblastosis fœtalis and normal fœtuses in utero (Hampson, 1928), carcinomatosis of bones (Waugh, 1936; Lucey, 1939) and in secondary hæmolytic anæmias associated with ovarian dermoid cyst, lymphosarcoma and pneumonia (Singer and Dameshek, 1941) and the hæmolytic anæmias due to sulphanilamide (Ham and Castle, 1940; quoted by Dacie, 1943). Between spherocytosis and increased fragility to hypotonic saline there is generally a more or less exact correlation (Gänsslen, 1922; Haden, 1934; Ponder, 1937; Castle and Daland, 1937).

This increase in fragility was thought by Troisier (1910, quoted by Chauffard *et al.*, 1912) to be due to the fixation of a hæmolysin on to the cells, an idea previously mooted by Widal *et al.* (1908). It has subsequently been brought forward again by Dameshek and Schwartz (1938*b*). Cases of hæmolytic anæmia with increased fragility of the red cells, in which circulating hæmolysin could be demonstrated, have been described frequently since the first reports by Chauffard and Vincent (1909) and Chauffard, Troisier and Girard (1912). Conversely, the anæmia and jaundice and the increased fragility and reticulocytosis of the red cells have been produced experimentally by the injection of exogenous poisons (Widal *et al.*, 1907*b*) and hæmolytic immune sera (Banti. 1913).

An abnormal function of the spleen has also been invoked as the cause of the increased hæmolysis. Minkowski and Chauffard both held this idea. Heilmeyer, from observations on cases of both acquired and congenital acholuric jaundice in which the spherocyte index returned to normal after splenectomy, concluded that there was no inborn error in the production of red cells and that the pathological red cells had been produced by altered splenic function. Whereas this may be true in some of the acquired cases which undergo complete clinical and hæmatological remission, either spontaneously or after splenectomy, it can hardly be so in the congenital cases, in which splenectomy usually leaves the increased red-cell fragility and in most cases the spherocytosis if not unaltered at least easily demonstrable (Haden, 1934; Lloyd, 1941; Dacie, 1943). Gripwall (1938), Fåhræus (1939) and Lloyd (1941) have attributed the hæmolysis to an excess of lysolecithin produced in the spleen by an undue circulatory stasis. On the other hand, Ham and Castle (quoted by Dacie) considered that circulatory stasis, acting on cells which were unduly susceptible to its effects, was the cause of the

lysis. The stasis was attributed to increased viscosity of the blood, slowing of the circulation, or, in the case of Dameshek and Schwartz's experiment of the administration of immune hæmolytic sera, to the presence of agglutinates. Dameshek and Miller (1943) have argued against erythrostasis pure and simple as the cause of the hæmolysis and noted that the hæmolysis does not occur unduly in heart failure, polycythæmia and other conditions where there is general circulatory stasis. They came to the conclusion that hæmolytic processes are probably due to various causes and not to a single factor like erythrostasis or the activity of a hæmolysin. However, one must conclude in the congenital cases, in which splenectomy effects a clinical cure and reduces the pigment excretion to normal (Watson, 1937 ; Barker,1938 ; Singer, Miller and Dameshek, 1941), that the spleen is largely involved.

The results of the physiological and serological experiments set out in this paper confirm the hypothesis of Widal *et al.* (1907c, 1908) that there are two different conditions having a different ætiology within the syndrome of acholuric jaundice.

(a) Congenital acholuric jaundice

Normal transfused red cells survive normally in recipients with this condition. Therefore indiscriminate blood-destroying processes cannot be at work. It appears that the transfused normal red cells are eliminated when they reach the end of their individual life-span. An abnormal hæmolysin or an over-active spleen or reticulo-endothelial system would hardly permit of this result unless its action was specific for the recipient's own cells. The fact that the red cells from cases of congenital acholuric jaundice, when transfused to a normal recipient, are eliminated unduly rapidly indicates that these cells are unduly sensitive to the normal destructive processes of a normal person and that therefore it is unnecessary to postulate abnormal hæmolysins or over-active blood-destroying processes in the patients themselves. The serological experiment, showing no agglutination by anti-human-serum serum of the washed red cells of these cases, also suggests that no immune hæmolysin or such sensitising agent is present.

Two current hypotheses are compatible with these findings and with each other. Whipple (1940-41), a proponent of an open circulation in the spleen (that is that the splenic pulp spaces provide the only link between the arterial and venous radicals), has suggested that the spherocytes cannot traverse the stomata of the splenic sinuses, whereas normal discoidal cells, because of their narrow diameter, can pass through with ease. Naegeli's original hypothesis of a congenital defect of the erythron, with slight modifications, can be made to account for the production of the spherocytic cells. The red cells are not discharged from the marrow as spherocytes (Henstell and Dameshek, quoted by Dameshek and Schwartz, 1940) and only develop the spherocytic form when mature. These cells, therefore, may well have an inborn defect which makes them unduly sensitive

Blood taken from these cases of acquired acholuric jaundice and transfused to normal recipients showed normal survival, even though the cells were apparently sensitised. The current conception of sensitisation of the red cell by an appropriate antibody is that the cell is then due for hæmolysis by the action of normal complement. It would appear that this is not necessarily so and that the process of sensitisation is a reversible one *in vivo*, so that under suitable conditions —in these instances in the circulation of a normal person—the immune antibody or hæmolysin can be washed off the cell, which reverts to its normal state. This conception is supported by the additional experiments quoted. Group A blood, sensitised by mixing the red cells with high titre anti-A serum—containing it is true a natural and not an immune antibody—survived normally after transfusion to normal recipients. Sensitisation *per se*, therefore, *in vitro*, as in this experiment, did not mean that the red cells were doomed to immediate hæmolysis *in vivo* under the action of normal complement. The same argument can be applied to the results of transfusing blood of cases of acquired acholuric jaundice to normal recipients.

In the patients themselves with acquired acholuric jaundice the red cells must be destroyed extremely rapidly. The excretion of bilirubinoid pigments is grossly excessive. There is obviously, therefore, some other factor present in these cases which completes the hæmolysis of the sensitised red cells. If it is the spleen or the reticulo-endothelial system it must be a pathologically potentiated spleen or reticulo-endothelial system, as this blood survives normally in a normal recipient. More probably, however, the hæmolysis is intravascular and not intracellular (*cf.* Fairley, 1940). In all 4 such cases where we examined the serum spectroscopically, Schumm's test for methæmalbumin (Fairley, 1941) was positive, showing intravascular red-cell destruction. There is, therefore, possibly some other abnormal potentiating substance in the plasma of these cases, as well as the immuno antibody or hæmolysin.

The presence of such an abnormal co-hæmolysin or abnormal complement may explain the anomaly in case 4, where the patient recovered completely but retained her sensitised cells. She also retained normal transfused blood normally. If, however, she had lost from her plasma this hypothetical co-hæmolysin the phenomena are explained.

The results of splenectomy in cases of acquired acholuric jaundice are never as dramatic as in congenital acholuric jaundice. In this series, cases 1, 2, 3 and 8 were ultimately considerably benefited ; the hæmolysis was slowed, allowing the regenerative processes to compensate for the destruction. In cases 6 and 7 splenectomy had little ultimate effect. It has been pointed out that case 7 might be considered one of secondary hæmolytic anæmia as there was extensive pulmonary tuberculosis. Singer and Dameshek have reported several cases of secondary hæmolytic anæmia with all the stigmata of

acquired acholuric jaundice. As acquired acholuric jaundice has been shown to be due to abnormal hæmolytic mechanisms, it is not improbable that all cases of acquired acholuric jaundice are really secondary hæmolytic anæmias, the primary lesion or metabolic disturbance being in most cases unidentified. Spontaneous cures are, therefore, not unexpected if the primary disturbance resolves; and if the primary disturbance or lesion can be identified, as in Singer and Dameshek's case of ovarian cyst, removal of this primary lesion results in cure of the anæmia.

Our term " acholuric jaundice " has been used throughout this paper to conform with the general British practice. It would be preferable to refer to the congenital form as familial hæmolytic anæmia (spherocytic type) and to the acquired form as acquired hæmolytic anæmia (spherocytic type). Ultimately, the acquired hæmolytic anæmias should be classified on their ætiology rather than on red-cell morphology.

SUMMARY

1. In cases of familial acholuric jaundice normal transfused red cells survive normally (Dacie and Mollison). Red cells of cases with familial acholuric jaundice on transfusion to normal recipients are eliminated unduly rapidly.

2. In cases of acquired acholuric jaundice normal transfused red cells are eliminated unduly rapidly. Red cells of cases with acquired acholuric jaundice on transfusion to normal recipients survive normally.

3. Red cells from cases of familial acholuric jaundice when thoroughly washed are not agglutinated by a rabbit anti-human-serum serum. Red cells of cases of acquired acholuric jaundice when thoroughly washed are agglutinated by this reagent.

4. The theories of the ætiology of acholuric jaundice, both familial and acquired, are discussed. It is suggested that the above results indicate that in the familial type there is a hereditary inborn defect of the erythron and that in the acquired type an abnormal " hæmolysin co-hæmolysin " system is in action, destroying both autologous and homologous red cells.

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OBSERVATIONS ON THE PULMONARY MACROPHAGE SYSTEM

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(PLATES CXLII-CXLV)

THE origin and nature of the phagocytes of the lung were an object of investigation by the earliest morbid histologists. Thus Slavjansky (1869) and von Ins (1878) indicated that these cells reached the alveolar spaces by diapedesis through the capillary walls, while Ruppert (1878) and Arnold (1882) traced their origin to the lining cells of the alveolar walls. Since that time many investigations have been made on this subject; reference should be made to the papers of Cappell (1929), Fried (1934) and Carleton (1934) for bibliographies. It is sufficient to state that three conflicting views are held on this question: (1) that the alveolar phagocytes arise from the monocytes of the blood by passage through the walls of the capillaries, (2) that they arise from the capillary endothelium, and (3)—the view held by the majority of investigators—that they are derived from cells lining the air sacs, either the so-called respiratory epithelium or cells of mesodermal nature, the "septal cells" of Lang. In view of those conflicting opinions, arrived at after numerous histological and experimental investigations, it would appear that little further progress would be likely without the use of new technical methods. Two methods are available for the selective demonstration of the macrophage system of the body: (1) staining with vital dyes, which has been extensively used in the investigation of this problem with few conclusive results, and (2) impregnation with ammoniacal silver or silver carbonate, which, although first used by del Río-Hortega and de Asúa (1921) over twenty years ago, has been almost completely neglected. This paper is mainly based on the use of the second method.

The technique used is the same as that employed by del Río-Hortega and others for the demonstration of the microglia and the cells so demonstrated are referred to by most authors as "microglia-like" cells. The literature on this subject is small. Following the original work of del Río-Hortega, de Asúa (1927) demonstrated similar cells in the spleen. Cone (1928) described them in degenerating areas of tumours and in the adventitia of vessels. Wells and Carmichael

(1930-31) showed cells in tissue cultures of chick tissues which were impregnated with silver carbonate and reacted to vital dyes in a similar manner to macrophages. Visintini (1931), by Bolsi's method, demonstrated cells resembling the microglia in cardiac and voluntary muscle and in the urinary bladder, Belezky (1931) " microglia-like " cells in the spleen, and Dunning and Stevenson (1934) showed that these cells (in the spleen and other tissues) responded to injury by forming rounded macrophages which absorbed vital dyes.

Dunning and Furth (1935) in further studies concluded that microglia and histiocytes elsewhere in the body form a single cell type. Robb-Smith (1938) described cells impregnated by del Río-Hortega's method in lymph nodes. Little attention appears to have been paid to the lung. Goyanès (1936) described cells, both branching and spheroidal, which were impregnated by del Río-Hortega's method in the lung of the rat, while El Gazayerli (1936) demonstrated spheroidal cells in the alveolar walls of the rabbit lung which were impregnated by Penfield's method. He concluded that these corresponded to Lang's " septal cells " and derived the alveolar phagocytes from them.

METHODS

In previous work with the silver carbonate technique, impregnation of frozen sections by standard methods for microglia appears to have been generally used. Owing however to the difficulty of handling frozen sections of lung and to the generally inferior results which, in the author's hands at least, have been obtained on material outside the nervous system, the following modification of Penfield's technique was devised. The method, although lengthy, has given generally reliable and often brilliant results on normal and pathological tissues, its chief drawback being poor penetration of the denser tissues. Of the standard techniques for microglia I have found the Weil-Davenport (1933) method applied to frozen sections by far the most satisfactory, provided that the concentration of the formalin reducing bath is brought down to 8 per cent.

(1) Fix tissues for 4 days in 5 per cent. formol-saline or preferably in formol-bromide (40 per cent. formaldehyde 15 c.c., ammonium bromide 2 g., distilled water 85 c.c.). Successful results may still be obtained after several months' fixation. (2) Cut tissues into blocks not more than 4 mm. thick and about 2 cm. square. (3) Wash blocks in running tap water for 7 hours. (4) Wash in 4 changes of distilled water for 2 hours. (5) Place in distilled water 50 c.c., with 10 drops of concentrated ammonia, and leave overnight. (6) Rinse in distilled water and place in the incubator in 5 per cent. hydrobromic acid for 8 hours at 37° C. (7) Wash for 1½ hours in distilled water (3 changes). (8) Place in 5 per cent. anhydrous sodium carbonate for 18 hours at 37° C. (9) Rinse for 1 or 2 minutes in distilled water. (10) Place in del Río-Hortega's strong silver sodium carbonate for 4-24 hours according to the density of the tissue. (For details of preparing this solution see Russell, 1939.) The pieces in the silver bath should be propped against the sides of the vessel and not allowed to slip to the bottom. (11) Rinse in distilled water. (12) Reduce overnight in 4 per cent. formaldehyde in distilled water. (13) Dehydrate in alcohol and embed in paraffin. (14) Cut sections at 15-30 μ from the surface of the block. (15) Tone the sections on the slides in 0·5 per cent. gold chloride. (16) Fix in hypo

and mount in balsam. The glassware used should be chemically clean and all chemicals including the formalin should be of " Analar " standards.

The Weil-Davenport method may be modified as follows. (1) Cut frozen sections of formalin-fixed tissue at 25 μ. (2) Wash in 2 changes of distilled water. (3) Place in ammoniacal silver solution for 5·15 seconds. (4) Transfer to 8 per cent. formalin in distilled water and gently agitate the section in the solution for 2 minutes. (5) Wash in distilled water. (6) Tone in 0·5 per cent. gold chloride. (7) Fix in 5 per cent. hypo, wash, dehydrate, clear and mount in balsam. Successful preparations should be a dark or golden brown colour before toning. A pale yellow colour indicates that the formalin reducing solution is too strong, a grey staining of the section, that it is too weak. Tissue that has lain in formalin for long periods may fail to be successfully impregnated. This is a rapid and reliable technique, but requires considerably more care in the interpretation of results than the silver carbonate method.

EXPERIMENTAL OBSERVATIONS

Results obtained in normal tissues by the silver impregnation techniques

Detailed accounts of the cells impregnated by these techniques in tissues outside the lung may be obtained from the references previously given. I have personally examined lymph nodes, spleen, kidneys, voluntary muscle, omentum and liver by the method described and by the Weil-Davenport technique. Two morphological types of cell are demonstrated which are affiliated through a large range of intermediate forms. For convenience of description they are referred to as type 1 and type 2 argyrophil cells.

Type 1 cells are abundant in lymph nodes and spleen (fig. 4) and in a slightly varied form in the liver. They are cells with a round or oval nucleus and cytoplasm which is prolonged axially and gives off lateral branches of variable length and thickness. Their cytoplasm in normal tissues is impregnated to a uniform black. While in many instances these cells closely resemble the microglia of the brain (fig. 1), the constancy of morphology seen in the nervous system is often lacking; thus simpler forms such as spindle, rod or unipolar cells may be observed.

Type 2 cells have rounded, regular cytoplasm and a nucleus that is either kidney-shaped or of similar form to type 1 but it is generally eccentrically placed in the cell. A large number of intermediate forms may connect these two types, a few of which are sometimes seen in normal tissues.

Owing to the confusion which exists in the nomenclature of cells of the reticulo-endothelial system, it is difficult to find accurate synonyms for these two cell types. The following, however, may be considered as approximate equivalents. *Type 1.* Fixed macrophage, resting wandering cell, adventitial cell of Marchand, clasmatocyte of Ranvier, rhagiocrine cell of Renaut, possibly some cells described as Rouget's pericytes, the melanophores of the skin (Visintini), the

Kupffer cells of the liver (fig. 2), the "trailer cells" of Buxton and Torry, and microglia of normal brain (fig. 1). *Type 2.* Amœboid wandering cell, polyblast of Maximow (supposed however to have origins other than from the fixed macrophage), large mononuclear phagocyte, "compound granular corpuscle", and histiocyte (fig. 3)— a term often used for cells of either type 1 or type 2.

Structure of the macrophage system in the normal adult human lung

This system, as shown by the silver carbonate technique, includes examples of both type 1 and type 2 macrophages in the perivascular, peribronchial and lymphatic tissues and in the alveolar walls of the lungs. The type 1 cells show a great variety of structure, from forms closely resembling the normal microglia of the brain (fig. 7) to spindle and rod-shaped forms (fig. 5). Many of the cells contain anthracotic pigment and in their typical situation round vessels and bronchi and in the interstitial tissues have long been recognised under the term "interstitial histiocytes". Although there is a well marked tendency for these cells to congregate in the perivascular and peribronchial tissues, they are not confined to these sites and may be found in the alveolar walls, and particularly in the alveolar angles, at considerable distances from them. They sometimes occur singly, sometimes in foci of considerable size. As a rule some are free from pigment and consequently not detectable by ordinary histological methods. Type 2 cells in the normal lung are present both in the sites occupied by type 1 cells and also in small numbers in the alveolar spaces. Those in the alveoli often tend to be closely applied to the alveolar walls. In such cells the depth of the silver impregnation is frequently much less than in the type 1 forms. Their cytoplasm, although approximating to the spheroidal form, is often markedly irregular and feebly impregnated, while apparently non-nucleated fragments which might be interpreted as portions of the cytoplasm of degenerated cells may be seen free in the alveoli. Cells which may be classified as intermediate varieties between types 1 and 2 (figs. 9-12) are also found in the alveolar walls and other sites where type 1 forms occur. The small lymph nodes in the lung substance also contain cells of both types, which possibly also give rise to free alveolar phagocytes. The anatomical estimation of the distribution of this system in the human lung is a matter of considerable difficulty, both because the large size of the organ requires a very large number of preparations for an accurate estimate and also because the effects of slight degrees of congestion and œdema in post-mortem material are difficult to assess. A tentative opinion may be expressed, however, that the greatest concentration of the macrophage system of the lungs is in the hilar regions, with a less pronounced tendency to a further concentration in the immediately sub-pleural zones.

Types of reticulo endothelial cells demonstrated by silver
impregnation techniques in human and animal tissues
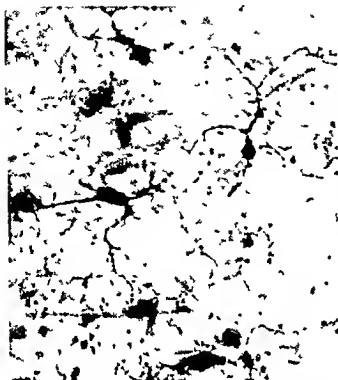
Fig 1—Normal microglia in human brain
Silver carbonate ×600
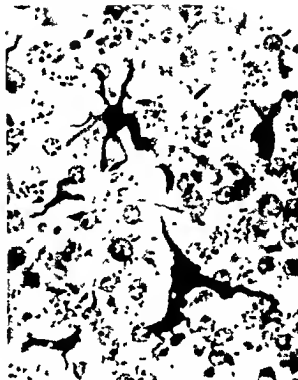
Fig 2—Kupffer cells in human liver
Silver carbonate ×550
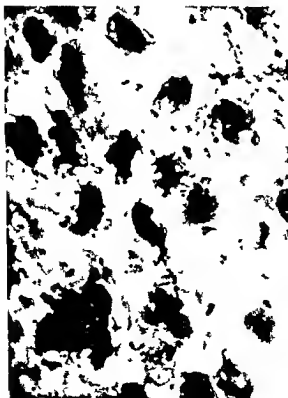
Fig 3—Proliferating histiocytes in
omentum of cat after repeated intra
peritoneal injections of vital dyes
Silver carbonate ×600

Fig 4—Type 1 histiocytes in Malpighian body
of human spleen Weil Davenport method
×200

PULMONARY MACROPHAGE SYSTEM
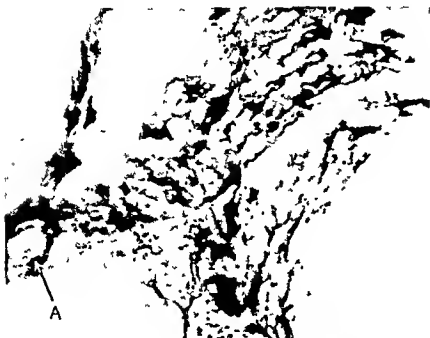
FIG. 5.—An aggregation of type 1 cells near a small vessel (A) in a normal human lung. Silver carbonate. ×525.

FIG. 6.—Lung from a case of subacute bacterial endocarditis. Numerous impregnated type 2 phagocytes are present in the alveolar exudate and numerous branching type 1 forms in the alveolar walls. Weil-Davenport method for microglia. ×135.
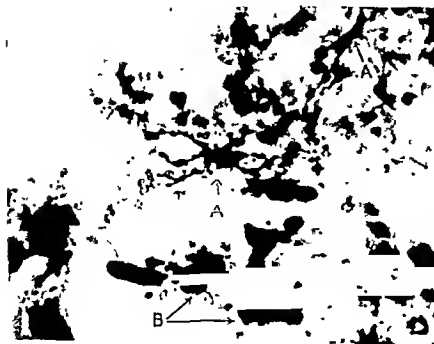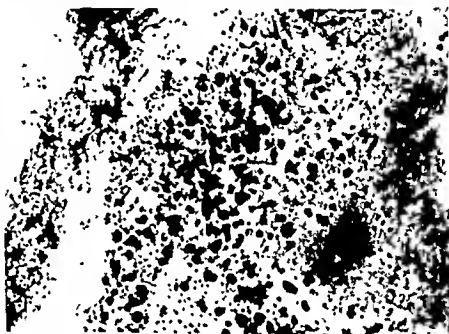
FIG. 7.—High power view of same lung. Two type 1 histiocytes (A) are present in the alveolar wall. A cluster of type 2 cells (B) is present in the alveolus. Weil-Davenport method. ×480.

The formation of the alveolar phagocytes in the human lung

Although in the normal human lung appearances suggestive of the development of free alveolar phagocytes from type 1 microglia-like forms may be seen, the process is not of sufficient frequency or clarity to justify such an assumption. Accordingly a study of pathological material was made to obtain further evidence. Examples of lobar pneumonia, bronchopneumonia, pulmonary infarction, carcinoma of the lung and pulmonary tuberculosis, in addition to lungs from cases of chronic congestive heart failure, were examined. In most of this pathological material little indication of the mode of formation was to be observed, although the alveoli were filled with impregnated cells of type 2 form. In some infarcts and in severe cases of congestive cardiac failure, however, a much clearer impression was gained. In the lung parenchyma immediately surrounding areas of infarction great numbers of the type 1 forms were found and these were often of an even more irregular and branched form than those seen in the normal lung. As the infarcted tissue was reached these gave place to forms in which the finer and sharper processes had disappeared, leaving an irregularly amœboid appearance. Finally a zone was reached in which ordinary type 2 spheroidal phagocytes were present, certain of which had wandered for some distance into the infarct, the whole process presenting a considerable resemblance to the formation of compound granular corpuscles in the margin of a cerebral softening. The detailed cell changes shown in figs. 8-13 represent essentially a retraction of the cytoplasmic processes into the cell body. Fragments of apparently non-nucleated cytoplasm, not forming part of the processes of any complete cell, may however be found scattered in the areas of cell transition, suggesting that the process involves some loss of cytoplasmic substance as well as its retraction into a spheroidal cell body. That macrophages may shed portions of their cytoplasm has been long known and accounts for the origin of Ranvier's term "clasmatocyte" (*cf.* also the recent work of Sabin, 1939). In the lungs of severe cases of congestive heart failure, apart from areas of infarction, foci showing a similar process were observed. These are intra-alveolar in situation and excited, as is well known, by the presence of extravasated red blood corpuscles, many of the impregnated cells containing hæmatogenous pigment. Similar intra-alveolar cellular foci were seen in a case of bronchopneumonia.

Mention may be made of the part played by the so-called respiratory epithelium, which is easily seen in silver preparations of pathological material. It consists of a layer of oval or cuboidal cells with round and usually central nuclei, attached to the alveolar walls and often desquamating in places as free rounded cells into the alveolar spaces. The cytoplasm of these cells is not impregnated by silver methods, and though they occasionally contain formalin pigment and sometimes minute black particles—probably silver granules—I have never seen

recognisable fragments of carbon or other form of ingested matter in them. It is theoretically possible, however, that they may acquire an affinity for silver and phagocytic powers only on becoming free in the alveoli; moreover human post-mortem material usually shows an uneven degree of impregnation of the macrophages. The appearances produced by this irregularity might be held as evidence supporting an origin of the macrophages from the alveolar epithelium. Attempts were therefore made to clarify this problem by animal experiments.

Experimental investigations on the origin of the alveolar phagocytes

In order to obtain further evidence of the early stages of formation of the alveolar phagocytes and of the response to vital dyes of the cells impregnated with silver carbonate, the following experiments on cats and rabbits were undertaken. The animals were divided into three groups.

Methods. **Group 1.** One cat and one kitten received daily intraperitoneal injections of 1 per cent. trypan blue. The cat received 6 injections of 10 c.c. per kg. body weight, the kitten 9 injections increasing from 7 to 20 c.c. per kg. body weight (total 31 c.c. of 1 per cent. trypan blue). The animals were killed 24 hours after the last injection and the lungs and other tissues fixed in formol-bromide and formol-saline. Portions of the lung and other tissues were impregnated by the silver carbonate technique, while paraffin sections were also made and counterstained with alum carmine. **Group 2.** Two cats and 4 rabbits were used. One cat received a single intratracheal injection of 1·5 c.c. of 1 per cent. trypan blue and was killed after 24 hours. The second cat received 4 daily intratracheal injections of 1 per cent. trypan blue, the dose being increased from 2 to 5 c.c. (total dosage 15·5 c.c.). This animal was killed 24 hours after the last injection. All injections were given under ether and chloroform anæsthesia. The rabbits each received an intratracheal injection of 4·5 c.c. of 1 per cent. trypan blue and were killed at intervals of 6, 12, 24 and 36 hours respectively after injection. The lungs of both cats and rabbits were examined by the same methods as in group 1, with, in addition, the use of the Weil-Davenport method for microglia on frozen sections of the rabbit lungs. **Group 3.** Five cats were used. They each received an intratracheal injection under ether and chloroform anæsthesia of 2 c.c. of 2 per cent. india ink, and were killed with coal-gas at intervals of $\frac{1}{2}$, 1, 2, 3 and 5 hours respectively after the injection. The cat killed at 3 hours had previously received in addition 6 daily intraperitoneal injections of 1 per cent. trypan blue at 20 c.c. per kg. body weight. Lung tissues were fixed and impregnated as in group 2.

Group 1. Macroscopically the abdominal viscera were stained deep blue, especially the liver. The lungs were a uniform pale blue. Silver carbonate impregnation showed a few cells of types 1 and 2 scattered through the lungs in the alveolar walls and peribronchial tissues. In ordinary paraffin sections stained with carmine, granules of trypan blue could be seen in a few cells in the lungs, the number of cells thus demonstrated being somewhat less than the number impregnated by silver carbonate. The amount of dye in the cells,

PLATE CXLIV

PULMONARY MACROPHAGE SYSTEM

FIG 8

FIG 9

FIG 10
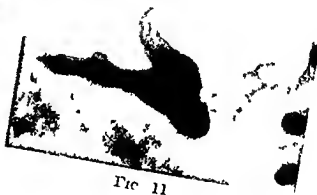
FIG 11

FIG 12

FIG 13

Figs 8-11. Cell in human lung containing a particle of ingested carbon (A)
Figs 9-12. Transitional forms showing gradual retraction of cytoplasmic processes
to form rounded type 2 cell (fig 13)

Silver carbonate   × 960

PULMONARY MACROPHAGE SYSTEM

Fig 14 —Lung of cat after receiving 4 daily intratracheal injections of 1 per cent trypan blue   The alveoli contain a number of phagocytes filled with granules of the dye   Alum carmine   ×660
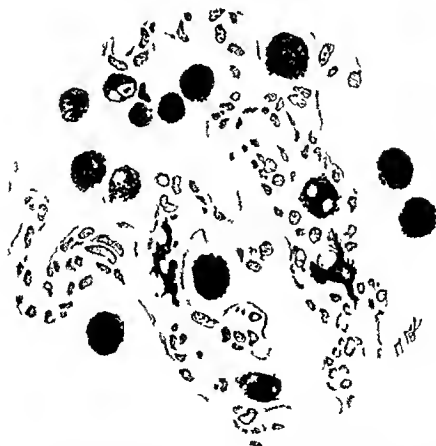
Fig 15 —Lung of same cat impregnated with silver carbonate   The impregnated cells contain the dye granules   Two type I cells are present in the alveolar wall   ×660

however, was small, and could not be seen through the silver impregnation. The omentum showed numerous heavily stained cells and in silver carbonate preparations granules of the dye could be seen in the impregnated cells. The Kupffer cells showed a moderate amount of intracellular dye. The comparatively feeble staining of the lungs after general vital staining is in agreement with most previous observations and is at least partly to be accounted for by the low affinity for vital dyes of the resting macrophages normally present in the cat's lung.

Group 2. The lower lobes of the lungs of both cats were stained deep blue. The first cat, which received a single intratracheal injection of trypan blue, showed a moderate number of type 2 cells, both in the alveolar walls and free in the alveoli. These contained granules of trypan blue and were all impregnated by silver carbonate, the dye being visible through the impregnation. The second cat, which received repeated injections (figs. 14 and 15) showed large numbers of type 2 cells in the alveoli, often clumped together. These were heavily stained with trypan blue and were all impregnated by silver carbonate, the trypan blue being clearly visible through the impregnation. In addition, a number of type 1 cells were present in the alveolar walls. In a few areas the appearances suggested direct formation of type 2 from type 1 cells.

The four rabbits showed numerous type 2 cells in the alveolar walls and alveolar spaces. Storage of dye by the impregnated cells became well marked at 12 hours and nearly complete 24 hours after injection. A number of type 1 cells were also present but showed little indication of transformation into type 2 forms.

Group 3. All these cats showed a considerable number of type 2 cells in the alveoli and alveolar walls. Many of the cells contained ink particles and, with the exception of cells too heavily loaded with ink for the silver carbonate impregnation to be visible, all the phagocytosis of ink appeared to be performed by impregnated cells. A slight increase of type 1 cells was present in some of the later animals in the series, but there was no evidence of direct production of type 2 from type 1 cells. The great proliferation that had taken place apparently arose from multiplication of type 2 forms. There was no evidence, in the animals of groups 2 and 3, of phagocytosis of carbon particles or of storage of vital dye by alveolar epithelial cells within the period of time for which these animals were studied. It should be emphasised that in attempting to determine whether all phagocytosis has been performed by impregnated cells, use should be made of impregnation of frozen sections as well as paraffin preparations. The occasional tendency of the latter to impregnate only in focal areas may make the interpretation of the results extremely difficult.

DISCUSSION

By the use of the silver impregnation techniques it has been shown that the normal lungs of man, the rabbit and the cat contain argyrophil cells of two types. Cells of similar types occur in many other tissues of the body and particularly in the organs that are the main sites of the reticulo-endothelial system. These cells store vital dyes and by their reactions in pathological processes appear to be identical with the macrophages. Some classification of the principal forms taken by these cells would seem to be desirable and for this reason the division into types 1 and 2 was made. It is unlikely, however, that these are the only changes in form which these cells can undergo, and this classification is only intended to apply to the pathological processes studied and to the methods of stimulation described in this paper.

Some consideration may be given to the functions in the lungs of the two types of cell described. The type 2 cell appears predominantly to be an active phagocyte towards bacteria, dead tissue in the lungs and inhaled foreign material. The type 1 cell, although it may store foreign matter, does not appear to be actively phagocytic and its capacity for staining with vital dyes is less marked. The multiplication of the cells of this system may take place in two ways. In the presence of mild stimulation, e.g. after the intratracheal injection of ink, direct division and emigration of the type 2 forms account for the considerable numerical increase that occurs. In severe and prolonged stimulation, however, as in pulmonary infarction, pneumonia and congestive heart failure, the type 2 cells appear to be derived from type 1 forms. There would appear to be a considerable time lag associated with this second process. While some multiplication of type 2 forms took place within 30 minutes of the injection of ink into the cat's lungs, no indication of the second mode of formation was to be seen in animals killed 5 hours after such injection. In infarction in the human lung the second mode of formation does not appear around fresh infarcts or areas of hæmorrhage, but only in those in which some degree of necrosis of the infarcted tissue has taken place. It is of interest in this connection that Russell (1929) observed that the formation of compound granular corpuscles from microglia in the brain of the rabbit was best seen between the 2nd and 4th days after injury.

The part played by the alveolar epithelium in cellular reactions in the lung remains obscure. Although these cells appear in considerable numbers in pathological material they seem to have no phagocytic properties (El Gazayerli) and in particular they lack the ability to store vital dyes. The question whether or not they form a true alveolar lining in the normal lung is beyond the scope of this paper; but if in fact such a lining exists, it must be provided by cells of this type and not, as suggested by Policard, by the processes of

the histiocytes, since the number of histiocytes normally present in the lungs is too small to cover more than a small area of the alveolar wall.

The monocytes of the blood appear to take a slight share in the cellular defence of the lungs, and while, owing to their small size, it is sometimes difficult to determine whether these cells are impregnated with silver carbonate or not, I have frequently seen cells with the nuclear characteristics of monocytes in blood clot in the vessels of impregnated lungs. The cytoplasm of such cells has never been impregnated. The possibility that, in accordance with Maximow's theories of inflammation, the monocytes and lymphocytes of the blood could be responsible for the formation of the alveolar phagocytes is worth close attention. The absence, however, of any degree of impregnation of these cells in the peripheral blood would indicate that they would have to acquire an affinity for silver after their emigration from the capillaries of the lung. I have never observed any evidence of transitional stages of this type and conclude that such a mechanism of macrophage formation, although there is considerable evidence of its occurrence in other organs, is not of importance in the lungs. Moreover, the experiments of Gardner and Smith (1927), in which a normal production of phagocytes was obtained in lungs perfused with Ringer's solution to remove all elements of the blood, would appear to eliminate the possibility of lymphocytes and monocytes being responsible for the production of the alveolar phagocytes. In the majority of pathological conditions in the lungs, apart from polymorphonuclear leucocytes and lymphocytes, the only cells taking part in the reaction are those of the type 1 and type 2 forms already described. In lobar and bronchopneumonia, however, mononuclear cells which are not impregnated by silver carbonate are present in the alveolar exudate ; some of these may be alveolar epithelium or degenerated type 2 cells which have lost their affinity for silver ; others are probably blood monocytes. In such acute infections, therefore, the monocytes of the blood probably play a part in the cellular defence of the lung.

SUMMARY

The use of the silver carbonate and ammoniacal silver techniques demonstrates two types of argyrophil cells in the lungs of man, the rabbit and the cat. These cells bear a close resemblance to the microglia of the brain and to the cells of the reticulo-endothelial system elsewhere in the body, both of which are demonstrated by the same methods. These cells store vital dyes and react in pathological processes to form the alveolar phagocytes. They are considered to be of mesenchymal nature and members of the reticulo-endothelial system. The possible part played by other types of cell in the defence of the lung is discussed.

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MYOCARDITIS IN FRIEDREICH'S ATAXIA

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(PLATES CXLVI-CXLVIII)

THIS communication is concerned with a peculiar form of chronic myocarditis which is associated with Friedreich's ataxia. The condition has attracted some attention in France but little notice elsewhere, yet the first record of myocarditis in this disease appeared in this country (Newton Pitt, 1886-87). In France several reports have been published in which the myocardial lesion has been more or less fully described. The literature has been collected and reviewed by Loiseau (1938). The myocardial changes have usually been ascribed to bulbar sclerosis involving the vagus nuclei and thus facilitating sympathetic overactivity (Philippe and Oberthür, 1901; Pic and Bonnamour, 1904; Guillain and Mollaret, 1932; Loiseau, 1938). But a few (Lannois and Porot, 1905; Lambrior, 1911) have argued that an unknown toxic agent was independently responsible for both the myocardial and nervous lesions.

The myocarditis described in the following four cases is similar in character to the kind already reported. It was first observed in case 1. Search through the records of the Bernhard Baron Institute yielded a second case (case 2) in which sections of the myocardium, in addition to other tissues, had been preserved. Cases 3 and 4 have recently been available for study through the kindness of colleagues. The pathological changes in the heart and central nervous system are so similar in the first three cases that these organs will be described collectively after the summaries. The distinctive features of the heart in case 4 will be described at the end of the section dealing with the first three.

SUMMARIES OF CASES

Case 1

Clinical. M. L., a girl aged 12 years, had developed normally up to the age of 3 years. Then, following an attack of measles and bronchopneumonia, she began to suffer from weakness and staggering, which increased in severity.

When first admitted to the London Hospital at the age of 8, she displayed marked ataxia with generalised hypotonia and absence of the deep reflexes. A bilateral extensor plantar response was obtained. There was no pes cavus, paralysis nor wasting. Lumbar puncture yielded normal cerebrospinal fluid which gave a negative Wassermann reaction. The heart was not enlarged; a loud systolic murmur was heard in the pulmonary area, the pulmonary second sound being slightly accentuated. The pulse was regular at 86-102.

Family history negative. Two sisters and one brother healthy.

After discharge from hospital she became worse and was readmitted in 1936, at the age of 11, with vomiting and abdominal pain following a blow on the nose. Surgical intervention was not required and improvement followed admission to the wards.

On examination. A thin, intelligent child with flaccid emotionless face. She had difficulty in starting and sustaining speech. Optic discs pale and well defined. Retinæ normal. Pupils unequal, the right being larger than the left, reacting poorly to light but normally on accommodation. Nystagmoid jerks on extreme lateral deviation. Other cranial nerves normal. Continual nodding movements of head. Great generalised hypotonia and few spontaneous movements of limbs. Tendon reflexes absent as on earlier examination. Gross ataxia of limbs. No sensory changes elicited.

Following discharge from hospital she went to a rural nursing home and died rather suddenly in the following year. A coroner's inquest was required and the following summary, based on the report of a pathological assistant from the Department, who attended the necropsy and secured certain organs for examination, is incomplete.

Summary of necropsy (P.M. 560/1935, Appendix)

Heart failure; chronic myocarditis; Friedreich's ataxia. Great hypertrophy of all chambers of heart, especially left ventricle. Muscle uniformly opaque greyish pink, with firm matt cut surfaces. No visible foci of fibrosis. A few recent subpericardial hæmorrhages over right auricular appendage. No pericarditis nor pericardial effusion. Endocardium and heart valves normal. Congestion of spleen. Congestion and advanced atrophy of centres of lobules of liver. Congestion and moderate fatty degeneration of right kidney. Recent and older anæmic infarcts in posterior border of kidney. Clear yellow ascites (about 1½ pints). Congestion, mucous catarrh and post-mortem digestion of stomach. Œdema of subcutaneous tissues of feet and lumbar region. Atrophy of thymus. Normal suprarenal bodies. Numerous caseo-calcareous glands in mesentery. Slight atrophy of middle and lower thoracic segments of spinal cord. Blurring of demarcation between grey and white matter at all levels of cord on section after fixation. No visible abnormality in brain. Healed pressure sores on posterior aspect of both heels and below right internal malleolus. No deformities. A wasted, red-haired freckled girl of about normal height for her age.

Weight of organs. Heart 312 g., spleen 57 g., liver 849 g., right kidney 85 g., brain 1157 g.

Case 2

Clinical. D. N., girl aged 10 years, was admitted to the wards of the London Hospital eight days before death on account of difficulty in walking and fatigue, with a tendency to fall. This had been developing for the previous five years, though she had attended school until one month before admission.

The parents were alive and well. Of seven siblings one, a brother, was known to have a deformity of the spine and had attended another hospital where the diagnosis of Friedreich's ataxia was made. The rest of the family appeared normal.

On examination. She was reported as of healthy appearance, and could speak and read well. There was marked equinus deformity. Irregular jerky nodding movements of the head were observed, also nystagmus on looking to the extreme left. Other eye movements were slow but full. The pupillary reactions were normal. No sensory loss was found. The knee- and ankle-jerks and the left plantar response were not elicited. The right plantar response was extensor. The heart was noted as "clear" by the clinical clerk. The pulse was regular at 90 on admission but rose to 130-140 on the day before death. The C.S.F. obtained by lumbar puncture gave a negative Wassermann reaction. No further examination of the fluid is recorded.

Intractable vomiting began on the second day after admission and she died on the eighth day.

Summary of necropsy (P.M. 278/1919)

Heart failure; chronic myocarditis; Friedreich's ataxia; aplastic anæmia. Hypertrophy of heart. Small patches of fibrous pericardial thickening near apex of left ventricle. Irregular opaque yellow and slaty grey areas throughout muscle of both ventricles. Right ventricle 0·7 cm. thick; left 2 cm. thick. Subendocardial fatty "tigering". Very slight atheroma. Œdema, congestion and fatty degeneration of liver. Fatty degeneration of kidneys. Small spleen. Purely fatty marrow with a few pink flecks in lower epiphysis and lower spongiosa of right femur; white fatty marrow in rest of medulla; very pale pink marrow in upper spongiosa and neck; yellow marrow in epiphysis of head and great trochanter. Fatty marrow (floats) throughout shaft of right humerus, for the most part pink in the upper 5/6ths; yellow marrow in epiphysis of head and tuberosity. Pale red marrow in ribs, sternum and vertebræ. No macroscopic changes in central nervous system recorded.

Body weight 37 lb. 2 oz. Body length 3 ft. 11 in. Heart 220 g., kidneys 142 g., spleen 28 g.

Case 3

Clinical. B. J., male, aged 21 years. His doctor stated that the patient had had severe attacks of vomiting when about 4 years of age. After that he was apt to fall about. At the age of 8 he was forbidden to join in sports on account of his heart, and at 11 he left school. He occasionally played cricket up the age of 17, though he was apt to fall when running. After 17 he began to need support in walking. He was employed as a shepherd. In June 1939, when he was 17, he was seen by Mr Pennybacker as an out-patient in the Nuffield Department of Surgery, Oxford. His general appearance was healthy.

On examination. Slight scoliosis and tendency to pes cavus. No swelling or atrophy of optic discs. Pupils and external ocular movements normal. Some ataxic dysarthria and a little titubation of the head. Cranial nerves otherwise normal. Gross ataxy of limbs, with surprisingly little weakness. No tendon reflexes obtained in arms or legs ; abdominal reflexes diminished and both plantar responses extensor. Defective postural and vibration sense in the lower limbs.

No neurological disease in the family. The only other child was said to be healthy.

The clinical diagnosis was Friedreich's ataxia. His doctor wrote that in May 1943 the patient was confined to bed with severe dyspnœa and tachycardia, the heart-beats and pulse being uncountable. He improved on digitalis and was able, after some weeks, to get about in a chair. He relapsed two months before death, which took place in May 1944, with similar symptoms. Ecchymoses appeared in the skin of the thighs and abdomen two weeks before death. No cardiac murmurs were recorded at any stage of his illness. By courtesy of Dr Thornton, necropsy was performed at Salisbury Royal Infirmary 36 hours after death.

Summary of necropsy

Heart failure ; chronic myocarditis ; Friedreich's ataxia. Slight generalised jaundice of skin, conjunctivæ and soft tissues generally. Considerable post-mortem degeneration of all organs. Deeply orange-stained ascites (a few ounces). Gross congestion of abdominal viscera, especially omentum, mesentery and retroperitoneal adipose tissue. Petechial hæmorrhages in smooth visceral pericardium, especially over right border of heart and auriculo-ventricular groove. Great engorgement of subpericardial adipose tissue. Great hypertrophy and dilatation of all chambers of heart. Right ventricle 0·6 to 1 cm. thick ; left ventricle 1·2 to 1·5 cm. thick. Focal congestion of otherwise clay-coloured, rather flabby myocardium. Aortic valve competent to water test. All valves normal. Organising thrombus occluding left auricular appendage. Considerable diffuse atheroma, sometimes constricting but nowhere occluding main coronary arteries and their branches. Slight atheroma of ring of aorta, but none elsewhere in aorta and its branches. Recent hæmorrhages in right parietal and visceral pleura and over lower lobe of left lung. A few foci of bronchopneumonic consolidation in deeply congested lungs. Recent infarcts in firm congested spleen. Jaundice, fatty degeneration and back-pressure congestion with paradoxical lobulation of liver. Moderate dilatation of gall-bladder. No obstruction in bile-ducts. A few foci of fat necrosis in congested pancreas. Cortical adenoma 0·5 cm. in diameter in otherwise normal suprarenal bodies. Old and recent infarcts in congested kidneys. Congestion of normal colloidal thyroid. Congestion of glandular thymus. Normal pituitary body. Middle ears dry. Post-mortem infection by gas-producing organisms of centrum ovale, but no further visible abnormality in brain. Slight general atrophy of spinal cord ; considerable in thoracic segments with flattening of dorsal columns. Poor demarcation

between grey and white matter of cord except in lumbar segments. Slight pitting œdema of moderately wasted lower limbs, and considerable of lumbar region. Bilateral pes cavus. Slight scoliosis in mid-thoracic region. A moderately nourished and otherwise well developed man.

Weight of organs. Heart 450 g., right kidney 120 g., thymus 18 g., suprarenals 15 g., spleen 122 g.

Case 4

Clinical. A. T., a male aged 23 years, was admitted to another hospital in November 1945 suffering from vomiting of three weeks' duration. He was said to have had Friedreich's ataxia since the age of 16. On examination he was very ill, with cold extremities and an irregular pulse of 100. His speech was slow and slurred and nystagmus was present. The knee-jerks were not obtained and he was unable to move his legs. Death took place within 24 hours of admission.

Necropsy was confined to the abdomen and thorax. Beyond the note that petechial bæmorrhages were present in the pericardium and on the viscera no further information about the case has been obtainable. The heart was received for examination after fixation in formalin.

Macroscopic examination of heart (weight 340 g.). The visceral pericardium was pale, smooth and mottled with petechial and larger (up to 0·5 cm.) recent hæmorrhages, mainly over the posterior surface and along the branches of the coronary arteries. All chambers were contracted. The valves, endocardium and coronary arteries appeared normal. The muscle was uniformly pale and firm and somewhat hypertrophied, the right ventricle being up to 0·9 cm. and the left up to 2·5 cm. thick. There was no obvious naked-eye abnormality apart from this. A few small flecks and streaks of atheroma were present in the aorta.

MICROSCOPIC EXAMINATION

The histological appearances of the spleen, liver and kidney in cases 1, 2 and 3, of the suprarenal bodies in cases 1 and 3, and of the thyroid, pituitary, thymus, lung and pancreas in case 3, have been incorporated in the summaries of necropsies.

Heart

Blocks from the right and left ventricle of all cases, of the left auricle in case 3, and of the sino-auricular node and conducting system in cases 1 and 3 were embedded in paraffin. Sections were stained with Ehrlich's hæmatoxylin and eosin, Weigert's iron hæmatoxylin and van Gieson's mixture, Weigert's elastic and neutral red, phosphotungstic acid hæmatoxylin, and (in case 4) Azan. Frozen sections stained by Sudan III for fat were prepared from the left ventricle in all cases, and from the right ventricle in cases 1, 3 and 4. A block of the left ventricle in case 3 was fixed in Bouin's fluid and sections were stained by Best's method for glycogen.

In *cases* 1, 2, *and* 3 the muscle everywhere shows great hypertrophy of the fibres (figs. 1, 2 and 3) as compared with a control specimen (fig. 4). Many fibres, distributed at random, are undergoing fatty degeneration. This is most pronounced in case 2. Small groups of fibres, and often individual fibres, are everywhere separated by a uniform increase of delicate collagenous tissue in which a moderate number of spindle fibroblasts are present. This fibrosis is least in case 2 and greatest in case 3. There is an uneven, rather scanty infiltration with small lymphocytes and fewer eosinophil and neutrophil leucocytes (figs. 5 and 6). Mast cells are also present in case 3. The infiltration is least in case 2 and greatest in case 1. The nuclei of the muscle fibres are large, often vacuolated and hyperchromatic, and irregular in outline. The longitudinal fibrils tend to occupy the peripheral sarcoplasm (figs. 6 and 7), the centre of the cell being occupied by granular cytoplasm in which, in cases 2 and 3, lipochrome pigment is often present. Cross striation is usually absent and, where present, is feeble. Fibres in which it is absent are often swollen and vacuolated. No glycogen was demonstrated in the left ventricle in case 3.

The Purkinje fibres of the node and conducting system are similarly separated by fibrous tissue and a sparse cellular infiltration. This fibrosis is conspicuous in the region of the sino-auricular node in case 3, but is only slight in case 1. Groups of fibres of the conducting system in the interventricular septum are flattened from ventricular dilatation, but the individual fibres do not appear to be altered.

In *case* 4 the appearances are in general similar to those already described, both ventricles being involved in extensive diffuse fibrosis, while the surviving muscle cells show marked hypertrophy (fig. 8). There are however important differences. Fatty degeneration of the muscle is extremely slight: it is confined to small groups of fibres beneath the endocardium of the right ventricle and to a few sub-pericardial fibres in the left ventricle. Acute stages of non-fatty degeneration and necrosis are present in small foci disseminated throughout the muscle of both ventricles (figs. 8 and 9). Here single fibres or small groups of fibres show either a coagulative eosinophil necrosis or a granular, slightly hæmatoxyphil change in the cytoplasm. The granules are sometimes brightly fuchsinophil and take a deep purple in phosphotungstic acid hæmatoxylin. Pyknosis of nuclei is occasionally seen but, for the most part, the fibres are shrunken and fragmented and the nuclei have disappeared. The foci are usually densely infiltrated with neutrophil leucocytes and fewer large phagocytic mononuclear cells, which are engulfing the necrosed fibres (fig. 9). Spindle-shaped fibroblasts are also present. No changes beyond slight atheroma affect the small arteries in the myocardium.

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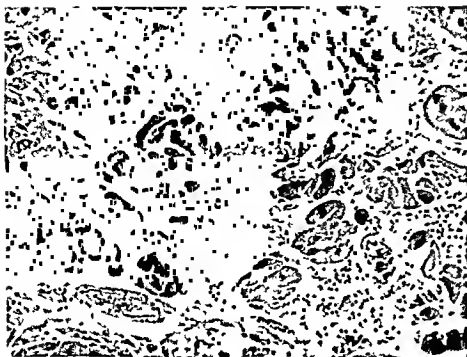
Fig 1 —Case 1 Left ventricle, showing cellular infiltration and wide separation of hypertrophied muscle fibres by fibrous tissue Hæmatoxylin and eosin. ×110.
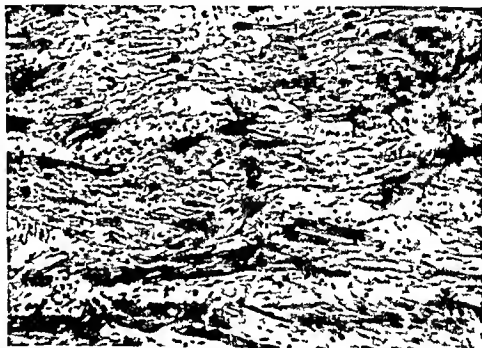
Fig 2 —Case 2 Left ventricle, showing hypertrophy of muscle fibres and sparse cellular infiltration Hæmatoxylin and eosin ×110
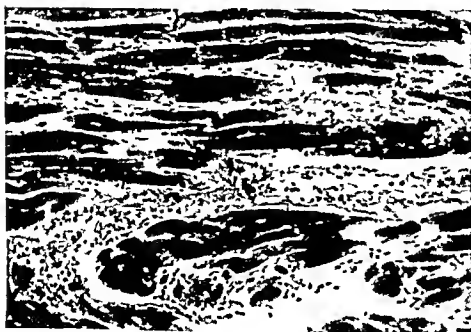
Fig 3 —Case 3 Right ventricle, to show hypertrophy of muscle fibres, cellular infiltration and increase of interstitial tissue Hæmatoxylin and eosin. ×110

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FIG. 4.—Control (P.M. 24, 1939). Female 27. Left ventricle. Hæmatoxylin and eosin. ×110.
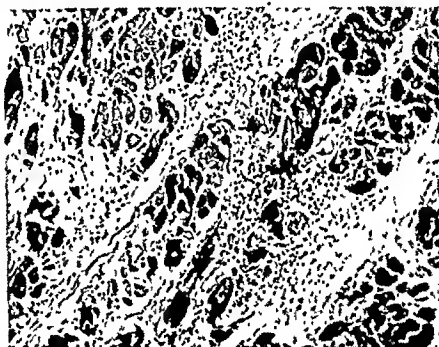
FIG. 5.—Case 1. Right ventricle, to show interstitial cellular infiltration. Hæmatoxylin and eosin ×110.
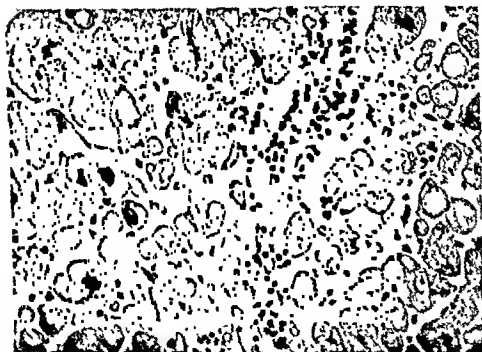
FIG. 6.—Same as in Fig. 5. Note distorted shapes of muscle nuclei and peripheral arrangement of myoglial fibrils. Hæmatoxylin and eosin. ×110.

Central nervous system (cases 1, 2 and 3)

Representative portions of cerebral cortex, basal ganglia, brain-stem (including both rostral and caudal levels of the inferior olives), cerebellum (including dentate nucleus), and spinal cord were embedded in paraffin. Sections were stained by the same methods as those used for the heart, with the addition of Loyez's hæmatoxylin. In case 2 additional blocks from the same levels and two further segments from the medulla oblongata were used for the Weigert-Pal method. In cases 1 and 3 additional blocks from the medulla oblongata and spinal cord were cut on the freezing microtome and sections were stained by Spielmeyer's method for myelin and Bielschowsky's method for neurofibrils.

No significant changes are present above the level of the medulla oblongata. The cerebellum, including the dentate nuclei, appears normal save for occasional focal loss of Purkinje cells, and ischæmic degeneration of neurones in the dentate nucleus (case 1).

In the medulla oblongata pallor of the dorsal spino-cerebellar tracts is slight in cases 1 and 3; no abnormality is present in case 2. The funiculi gracilis et cuneatus show considerable loss of myelin in cases 1 and 3; they were not sectioned in case 2. A special search was made for histological changes in the region of the dorsal motor nucleus of the vagus and the nucleus ambiguus. No changes beyond varying degrees of chromatolysis, affecting a minority of the neurones, are present in any of the cases. There is no apparent numerical loss of neurones and no sclerosis or demyelination in these regions. In case 3 there is extensive degeneration in the nuclei of the descending root of the fifth nerve and, less marked, in the adjacent nuclei gracilis et cuneatus. A similar but less pronounced degeneration at this level is present in case 1.

In the spinal cord all cases show great demyelination and gliosis of the posterior columns throughout all segments, especially the column of Goll. A less pronounced and more variable degeneration is present in the dorsal spino-cerebellar and crossed pyramidal tracts: it is maximal in the thoracic segments and diminishes caudally in cases 1 and 3, but gradually increases caudally in case 2. In case 2, moreover, the involvement of the lateral columns is unilateral in the cervical region; in the thoracic segments this disparity affects the pyramidal tract only, and in the lumbar cord the degeneration is symmetrical. The grey matter is unaffected, with the exception of Clarke's column, which shows great atrophy in all cases, but again this is unilateral in case 2. There is no inflammatory cellular infiltration at any level of the cord or its meninges. The blood vessels appear normal.

DISCUSSION

The myocarditis in these four cases appears to be of a chronic progressive character. Although fatty degeneration was present in cases 1-3 and in several of the previously reported examples, it would seem, if case 4 may be accepted on the scanty available

data as a true example of Friedreich's ataxia, that destruction of the muscle takes place through a focal, piecemeal, coagulative necrosis of the fibres. As a result the fibres are replaced ultimately by collagenous tissue and the surviving muscle undergoes a compensatory hypertrophy. The histological appearances suggest that this process continues over a prolonged period until a stage of heart-failure develops, and that the severe fatty degeneration is a terminal phase. Newton Pitt described, in his case, a focal granular degeneration of the muscle fibres, and Pic and Bonnamour noted the remains of muscle fibres in process of disappearance. But in neither was the pathology of this lesion more fully described, nor is there any mention of an associated cellular infiltration.

On the neurological side the clinical and histological features of the first three cases are characteristic of Friedreich's ataxia. In case 2 a second member of the family was similarly affected. Medullary degenerations were limited to the rostral extensions of the ascending sensory tracts of the posterior columns, the dorsal spino-cerebellar tracts and, in case 3, the nuclei of the descending root of the fifth nerve. Examination of the vagal nuclei revealed no significant change in any instance; the neurones were not numerically reduced nor were the nuclei involved in either sclerosis or demyelination. The degrees of chromatolysis present affected other neurones also and were attributable either to the heart failure or post-mortem degeneration. The precise origin of the efferent fibres responsible for the innervation of the heart is attributed by most to the dorsal motor nucleus of the vagus, but by some to the nucleus ambiguus (Greving, 1928). Malone (1913-14) couples the nucleus ambiguus with the supply to striated muscle and agrees with Molhant in deriving the cardiac fibres from the middle portion of the dorsal motor nucleus. The issue, however, is immaterial to the present thesis, since neither nucleus is demonstrably affected. It is impossible, therefore, to agree with those who have attributed the cardiac lesion to degeneration of the vagal nuclei and sympathetic over-action, nor is it easy to conceive how a myocarditis of the kind described could result from this disordered control. The changes in the myocardium suggest the interaction of a toxin, but there is no evidence as to its identity. Interstitial non-purulent myocarditis is recognised as a sequel to scarlet fever and influenza; a sporadic form known as Fiedler's myocarditis may be related to respiratory infection (Covey, 1942). It is also of interest that a myocarditis similar to that in case 4 is described by Follis (1942) in rats suffering from potassium deficiency. In this deficiency, however, the kidneys also showed severe tubular degeneration and necrosis. In these cases of Friedreich's ataxia it is remarkable that no inflammation was found in the other organs; no focus of possible infection was disclosed at necropsy. In favouring a toxic origin for the myocarditis it is important to consider the possible relationship between this and the degeneration of the central nervous

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FIG 7—Case 2 Right ventricle, showing granular sarcoplasm in centre of fibres and peripheral distribution of fibrils Phosphotungstic acid hæmatoxylin ×250

FIG 8—Case 4 Left ventricle, showing interstitial fibrosis, hypertrophy of muscle fibres and a focus of cellular infiltration Hæmatoxylin and eosin ×110
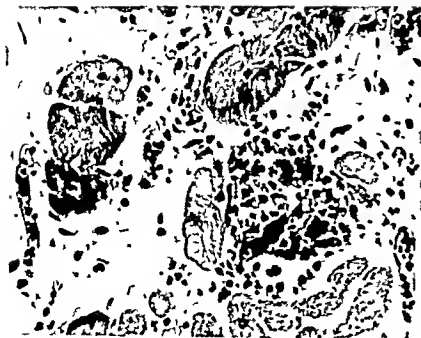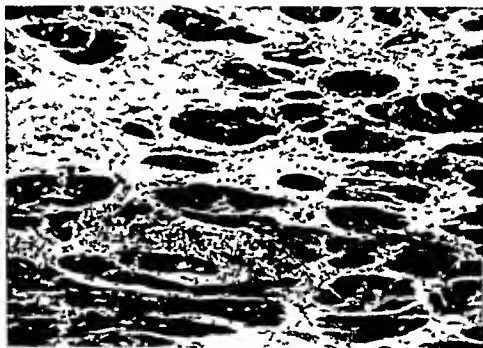
FIG 9—Case 4 Focus in right ventricle showing necrosis of fibres and cellular infiltration Earlier degeneration of a fibre without infiltration seen to left of centre Hæmatoxylin and eosin ×300

system. That it is not fortuitous would be suggested by the present four cases alone. Loiseau's series, collected from the literature, includes sixteen cases in which necropsy was performed. Ignoring three of these, in which a valvular lesion of the heart was demonstrated, there was hypertrophy of the heart in six, dilatation in four and fatty degeneration in six; one heart was described as small and one as normal. The incidence of myocarditis cannot be estimated because the information is often meagre and confined to the macroscopic appearances. In case 4 of the present series the heart was not obviously abnormal to the naked eye. An interstitial myocarditis similar to that described in this paper has, however, been recorded in four cases (Newton Pitt; Pic and Bonnamour; Lannois and Porot; and Lambrior), that is, in all examples that have been examined under the microscope since Friedreich (1863), who found extensive fatty degeneration in his cases 1 and 3, with thickening of the ventricle in case 3. Irregularity of the heart's action in Friedreich's ataxia has been noted clinically from the time of Friedreich onwards, and has been the subject of several communications in the French journals, although this aspect of the disease has been neglected in most neurological text-books. Evans and Wright (1942) examined 38 cases by the electrocardiograph; they report conspicuous or significant changes in 12 and slight changes in 10. They concluded that the disease may be as much an affection of the heart as of the nervous system. The incidence of cardiographic changes seemed in no way to be related to age or sex, or to the time of onset of the disease. On the other hand, it is of interest that there was an association between the abnormal cardiographic changes and the presence of a family history. Moreover the affected members of the one family tended to show the same type of change. While the full significance of these observations cannot at present be appraised they seem to point one way, namely to the inheritance of a lethal gene which affects both the heart and the central nervous system.

SUMMARY

Four cases are reported in which Friedreich's ataxia was associated with a chronic interstitial myocarditis, with pronounced cardiac hypertrophy in three.

Examination of the medulla oblongata failed to show any histological abnormality in the region of the vagal nuclei.

It is argued that the myocarditis is of toxic origin and, in view of the known association between the nervous and cardiac disorders in Friedreich's ataxia, it is probable that the same agent is responsible for both lesions.

I wish to thank Dr A. H. T. Robb-Smith and Dr Douglas Thornton for their help in obtaining the necropsy on case 3 and Dr Baynton Forge for his clinical notes on this case. The heart in case 4 was kindly sent by Dr R. Kempthorne from the North Middlesex County Hospital. I am indebted to Mr A. John King for his help with the photomicrographs.

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THE BLOOD PICTURE AND PLASMA PROTEIN LEVEL FOLLOWING INJURY *

JANET VAUGHAN, MARGOT THOMSON and MARY DYSON

The N.W. London Blood Supply Depot

OBSERVERS who have followed the blood picture of air-raid and battle casualties have been struck by the fall in hæmoglobin level that occurs in the first fortnight after injury. In the case of burns this fall in hæmoglobin is associated with changes in plasma protein (Vaughan, 1943 ; Taylor *et al.*, 1943 ; Cuthbertson, 1944 ; Anderson and Semeonoff, 1944). The degree of anæmia that may develop appears to be out of proportion to the amount of blood lost at the time of the incident. Apart from burns few accurate and detailed studies have, however, been made of the complete blood picture in such patients. As a first step to an understanding of the ætiology of this anæmia and hypoproteinæmia, a study of the peripheral blood picture and protein levels in various types of trauma was planned. Experience has proved that it is not easy to carry out such an investigation in an entirely satisfactory way under war-time conditions. Air-raid casualties are liable to suffer from multiple injuries, often affecting the arms, so that the withdrawal of adequate blood samples is difficult. Patients are constantly transferred without warning to other hospitals and much time is wasted in the pursuit of such patients whose investigation is not completed. Further, a sudden rush of casualties needing immediate therapeutic help may hold up all investigative work for several days. It has also proved difficult to train the staff in many different hospitals to collect the necessary samples of urine required for pigment analysis.

METHODS

Red- and white-cell and reticulocyte counts were made by methods already described (Price-Jones, Vaughan and Goddard, 1935). White-cell counts were made on blood withdrawn from the ear; other estimations were made on venous blood, heparin being used as anti-coagulant. When in certain cases of head injury it was impossible to obtain samples from the ear, venous blood was used. Immediately after the blood had been mixed with heparin a sample was withdrawn into a diluting pipette and mixed with diluting fluid. Differential counts were made on films stained by Jenner's stain. Red-cell fragility in saline was estimated by the method of Dacie and Vaughan (1938).

Hæmoglobin was estimated with apparatus conforming to B.S.I. specification *no. 1079, using* apparatus checked by the National Physical Laboratory and following the instructions laid down by the Committee on Hæmoglobin Surveys of the Medical Research Council (1945).

The sedimentation rate was estimated by Wintrobe's method (Wintrobe and Landsberg, 1935).

Urobilin in the urine was estimated by *Schlessinger's method* (Stitt *et al.*, 1938), serum bilirubin by the method of Haslewood and King (1937) and serum and plasma protein by the micro-Kjeldahl technique (Dyson and Plaut, 1943). Differential proteins were estimated by the method of King *et al.*, 1942. It was hoped, in planning the investigation, to make at least daily observations on all patients. This proved impossible owing to the dispersal of casualties to hospitals in different parts of the country. In future work it is important to make frequent observations, especially in the first 48 hours.

OBSERVATIONS

The 14 patients studied may be divided into three groups: (I) accidents other than those due to enemy action; (II) industrial accidents in which superficial burns were associated with fractures; (III) accidents due to enemy action.

Brief clinical details of each patient, with age and sex, are shown in the table (p. 753). The date on which the injury occurred, even if this took place late at night, is considered for purposes of discussion and in the preparation of the diagrams as the first day.

Group I.   Accidents other than those due to enemy action

In this group are included 5 patients with simple *fractures* and one with a large hæmatoma of the scalp.

Hæmoglobin and red cells (fig. 1). In one patient (case 1) with only a mild injury there was no significant alteration in Hb. during the 8 days the patient was under observation. There was a slight fall in red cells from 5,700,000 to 5,200,000 per c.mm. In cases 2 and 3 there was a slight rise immediately after admission, followed by an appreciable fall reaching a maximum between the 8th and 10th days. In case 2, an old man with bronchitis, there was no appreciable rise during the 21 days of observation. In case 3 there was slight improvement until a further operation became necessary. This was followed by a further fall in Hb. of 18 per cent., the lowest figure being reached on the 9th day after operation. Some blood was lost at the time but there was no subsequent bleeding. In cases 5 and 6 there was a fall in hæmoglobin which reached its maximum on the 8th and 6th day respectively. In case 5 there was then an uninterrupted rise. In case 6, following an operation, the rise which had started was interrupted by a further fall: this reached a maximum 8 days after operation. There was then a slow improvement. Case 4 was not seen until 8 days after the accident. From that date there was a rise in Hb. The changes in Hb. level were associated with

changes of the same order in the total red-cell count. Four out of 6 cases therefore showed a definite and gradual fall in Hb. in the 10 days following injury. In the fifth case—not seen until the 8th day—
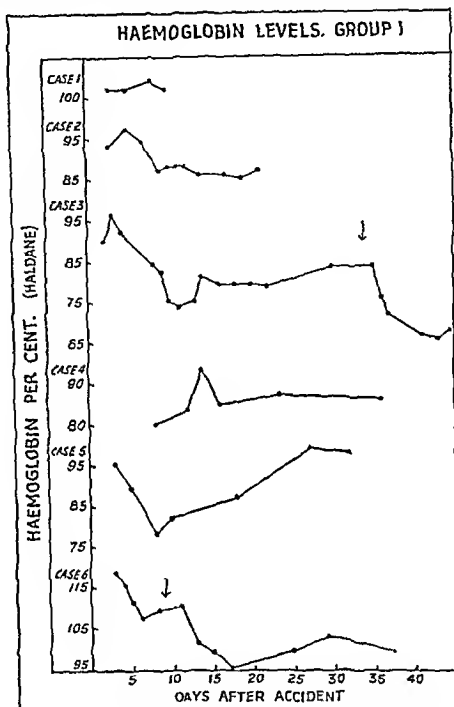
Fig. 1.—Hæmoglobin levels. Group 1.

it can be presumed that such a fall had occurred, since there was a rising Hb. level. In the sixth case, where the injury was slight, the Hb. level was unaffected. No abnormality was noted in the appearance of the red cells in stained films.

Reticulocytes (fig. 2). No change in the reticulocyte count was seen in case 1, where the injury was slight. In cases 2, 5 and 6 there was a rise within normal limits associated with the rise in Hb. In case 3 the count was in the low range of normal for the first 9 days *after the accident* and then started to rise, just before there was a slight improvement in Hb. A figure within the upper limits of the

normal range was then maintained until, following a second operation, there was a further rise associated with the presence of both hæmorrhage and sepsis. In case 4 there was a rise in reticulocytes to a figure as high as 4·2 per cent. on the 12th day, followed by a fall to the normal range on the 18th day. This high figure may be associated with the fact that the patient had previously been on a most inadequate diet.
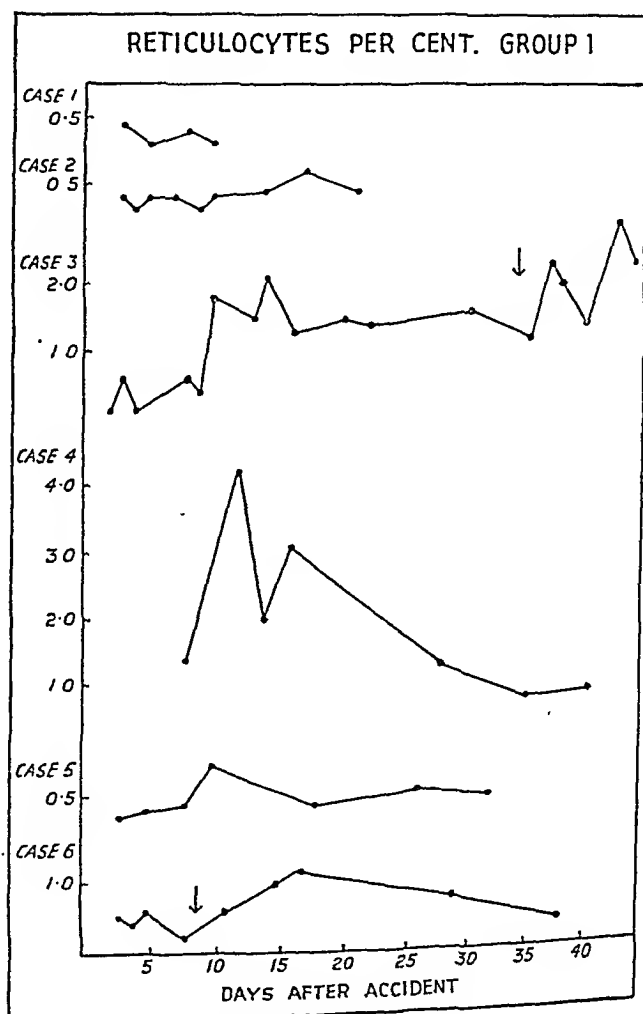
FIG. 2.—Reticulocyte counts (per cent.). Group I.

Mean corpuscular volume. The mean corpuscular volume of the red cells remained within normal levels except in cases 2 and 3, where, during the early phase of the anæmia, there was an increase, the maximum observed being 100 c.μ. and 97·1 c.μ.

Red-cell fragility. In case 5 observations on red cell fragility were made on the 5th day after injury, when the red cell count and Hb.

were falling, and again on the 10th and 18th day   No significant alteration in median corpuscular fragility was noted

TABLE

Clinical details of cases

Case no	Sex	Age	
1	M	23	Hæmatoma of right malar and temporal region Hæmorrhage from rt ear No fracture Afebrile
2	M	67	Fracture of neck of femur Febrile cold on admission 3 days later, sulphamethazine Extension applied 3rd day
3	F	34	Fracture of neck of femur 3rd day, 3½ inch flanged nail inserted Sixth day, Smith Peterson pin hæmatoma under wound 19th day, injured her leg moving in bed 32nd day, operation removal of old pin insertion of Smith Petersen pin bone graft from left fibula to femoral neck 33rd day, rising temperature and pulse sulphamethazine 32 g hæmatoma in wound, which drained well 92nd day, œdema and tenderness over part
4	F	52	Fracture of neck of femur Old disseminated sclerosis Insertion of Smith Petersen pin 5th day, febrile throughout Sulphathiazole on admission and 16 days later bed sores
5	M	39	Fracture of upper half left femur large hæmatoma Steinman pin ~~~~~ ays, max 101°
6	M	36	large hæmatoma Febrile 12 days, max 100 4° Sulphamethazine 7th day
7	M	42	Superficial petrol burns of face, right arm and hand compound fracture, torn end of radius and ulna right metacarpo phalangeal joint opened and tendons cut 1 pint of serum sulphathiazole febrile until 6th day
8	M	37	Fracture of pelvis penetrating wound rt thigh with some hæmor rhage superficial burns both arms and rt hand (?) ruptured spleen (no satisfactory evidence of this) 1 bottle plasma followed by saline or glucose saline drip for 6 days 5th day, increasing fever up to 101 8° 8th day, sulphathiazole and two pints fresh blood 13th day, 2 pints fresh blood 28th day, blood aspirated from chest
9	M	17	Multiple lacerations of neck, chest, abdomen and legs foreign bodies in scalp fracture of fibula febrile Sulphathiazole 2nd day 21st day, persistent discharge from wound of rt thigh Tab M & B 760 29th day, operation to open up sinus in rt thigh
10	M	19	Compound fracture of skull exploratory trephine lacerated leg and thigh 4th day sulphathiazole febrile until 13th day 21st day fever Spinal fluid tested and under pressure (?) meningitis
11	M	18	Lacerated eye lacerations of left hand exposing metacarpo phalan geal joint lacerations of abdominal wall and rt thigh Fracture of rt tibia Foreign bodies in scalp 5th day, febrile, sulphathiazole 18th day, sudden rise of temperature (?) influenza
12	M	18	Multiple lacerations of head arms and legs right malar fracture Swinging temperature, max 100° Local sulphanilamide, tab sulphathiazole 8th day
13	M	17	Multiple abrasions fractured pelvis hæmatoma over rt iliac crest Febrile (up to 100°)
14	M	39	Puncture wound of left face large hæmatoma compound fracture of rt radius cut into elbow joint wound of left frontal region puncture wound of left chest Perforation of body of stomach sutured on admission No transfusion Sulphathiazole Febrile 1 week Ulcer diet high protein intake 56 86 g for 20 days then full diet

White cells   No dramatic change in the total white-cell count was observed   Case 3 had, when first seen, a count of 14,200 per c mm This rose to 17,800 per c mm during the first period of anæmia,

but no rise was noted in the second period of anæmia. With one exception, however, the count at the end of observation was lower than that seen at first, though both figures were within normal limits. No abnormal cells were seen at any time and the differential counts presented nothing unusual.

Serum bilirubin. This was followed in five instances. No figure outside the normal range was found (Vaughan and Haslewood, 1938). In 3 instances the level at the end of the period of observation was lower than at the beginning (cases 1, 5 and 6). In one there was a slight rise followed by a fall (case 3). The second operation in this patient was followed by a further rise. The changes, however, were slight.
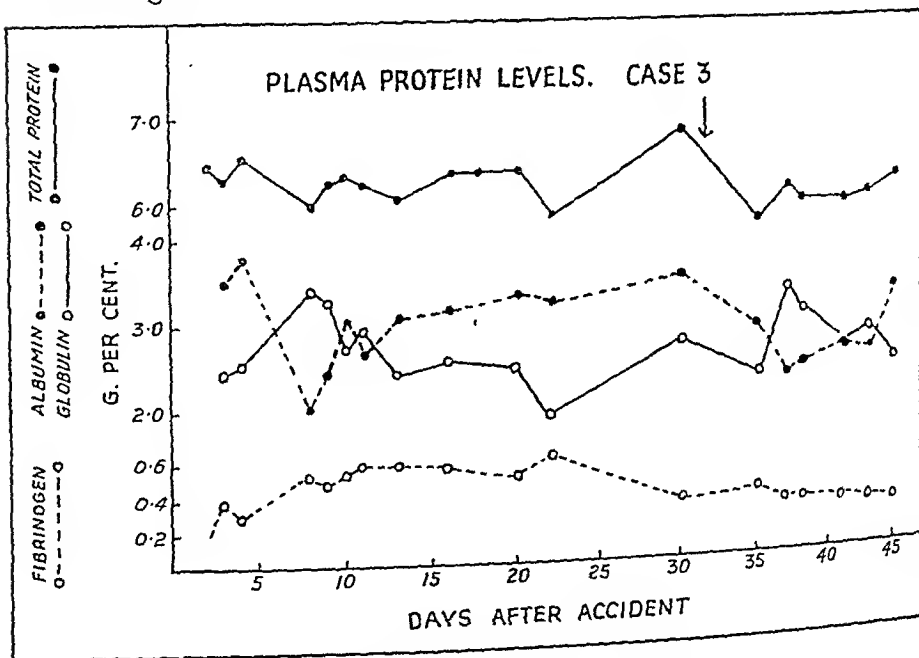
Fig. 3.—Plasma-protein levels. Case 3.

Plasma protein. Total and differential estimations of plasma proteins were made in cases 3 and 6. In the other four cases total protein only was estimated. Cases 2 and 5 showed an immediate fall in total protein followed by a slow rise. In case 1, where the rise was slight, the level on the 8th day was 7·03 g. compared with an initial figure on the 3rd day of 6·14 g. It is possible therefore that an immediate fall had followed injury. In case 4 there was an initial low level of 5·16 g. on the 8th day, followed by a slow rise to 5·82. In case 3 (fig. 3) there was no gross change in the level of total plasma protein during the first 20 days following the accident; there was then a considerable rise until the second operation, when there was a further sharp fall. The differential plasma-protein estimations show, however, that the injury was followed by a rapid fall in albumin

from 3·75 to 2·0 g. on the 8th day and an associated rise in both globulin and fibrinogen fractions. The rise in these two was sufficient to mask the fall in albumin. Though the fibrinogen remained high the globulin, which reached its peak on the 8th day, then slowly fell to normal levels, the altered ratio being maintained for 5 days. The second operation was followed by a fall in total protein, which was apparent on the third day. The albumin, however, reached its lowest level on the 5th day after operation, when the total protein appeared to be rising owing to a marked increase in the globulin fraction. This alteration in the albumin-globulin ratio was maintained
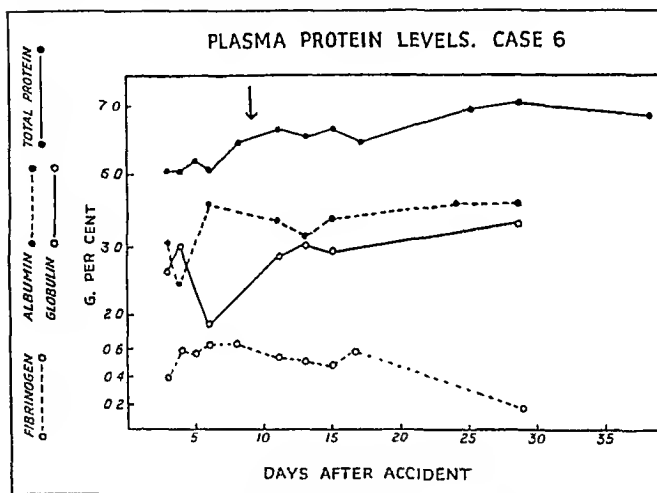
Fig. 4.—Plasma-protein levels. Case 6.

for 7 days. In case 6 (fig. 4) the total plasma protein rose slowly during the period of observation from a level of 6·19 to 6·89 g. The first observation was made on the 3rd day, so it is possible that the initial level may have been higher. The reversal in the albumin-globulin ratio noted in case 3 was also apparent. On the 4th day there was a fall in albumin, compensated for by a rise in both globulin and fibrinogen.

Sedimentation rate. With one exception (case 6) the sedimentation rate was raised in all cases when first seen and only returned to normal while under observation in case 1, who was but slightly injured.

Urobilin. Collection of specimens in this group of patients proved difficult and regular daily tests were impossible. No urobilin was

found in the specimens examined from cases 5 and 6. It was not looked for in case 1. A trace was found in case 2, and in case 3 on the occasion of the first injury. On the occasion of her second injury large amounts were present. Considerable amounts were also found in case 4 on more than one occasion.

Group II. Industrial accidents

Case 7. This patient had superficial burns of both forearms and hands and a fractured pelvis. On admission to hospital he received one bottle of plasma and in the following 6 days, at the wish of the surgeon, he was given at least 6 litres of saline or glucose-saline, as intra-abdominal bleeding was suspected without any definite evidence. In view of his blood picture it was then suggested that blood was indicated and two pints were given with dramatic improvement in the general condition.

Red cells and hæmoglobin. When seen shortly after injury the red-cell count was 4,160,000 and the Hb. 84·5 per cent. Six days later the red-cell count had fallen to 2,610,000 per c.mm. and the Hb. to 54 per cent. Stained films showed anisocytosis and polychromasia. Following a transfusion of fresh blood the count rose to 3,500,000 and the Hb. to 66 per cent., at which levels they remained, approximately stationary, for 12 days: then the red cells gradually rose to 4,300,000 and the Hb. to 82 per cent.

Reticulocytes. A maximum reticulocyte count of 2·4 per cent. was observed immediately before transfusion. After transfusion the count remained between 10 and 2·2 per cent. for some days and then fell to below 1·0 per cent.

Mean corpuscular volume. This was not measured, as venous samples could not be obtained owing to the injury to the arms.

Colour index. This remained in the neighbourhood of 1 throughout observation.

Urobilin. Marked amounts were present in the urine from the 3rd day after injury for 34 days.

Case 8. This patient, following a petrol explosion in his place of work, was admitted to hospital with superficial burns of his face and right arm and hand, a compound fracture of the humerus, and compound fractures of the lower end of the radius and ulna. The right metacarpo-phalangeal joint was open. Before being taken to the theatre he was given two bottles of reconstituted serum.

Red cells and hæmoglobin. The day following the accident the red-cell count was 4,450,000 per c.mm. and the Hb. 90·5 per cent. Ten days later the red cells had fallen to 3,750,000, the Hb. to 67·5 per cent. From this date there was a slow rise in the following 35 days to a red-cell count of 5,100,000 and a Hb. of 94·5 per cent.

Reticulocytes. During the period of anæmia the reticulocyte count was in the neighbourhood of 1 per cent. and fell with recovery to figures ranging between 0·2 and 0·4 per cent.

Mean corpuscular volume. With the exception of one observation of 77·5 c.μ, the mean corpuscular volume remained within normal limits.

Fragility. Red-cell fragility was observed on 6 occasions. As might be expected there was a slight shift in the curve to the right with increasing anæmia and return to the left with recovery. Median corpuscular fragility was never increased but there was a slight tail outside normal limits on one occasion.

Serum bilirubin. When first seen the serum bilirubin was 0·75 mg. As the anæmia improved the figure fell to the neighbourhood of 0·2 mg.

Serum protein. There was a fall in serum protein from 6·46 g. when first

estimated the day after the accident to 5·42 g. on the 10th day. There was a subsequent rise to 6·5 g.

Sedimentation rate. This was increased throughout the period of observation.

Urobilin. This was not seen in the urine, though repeatedly tested for.

Group III. Accidents due to enemy action

Six patients are included in this group. They all had multiple injuries typical of air-raid casualties.
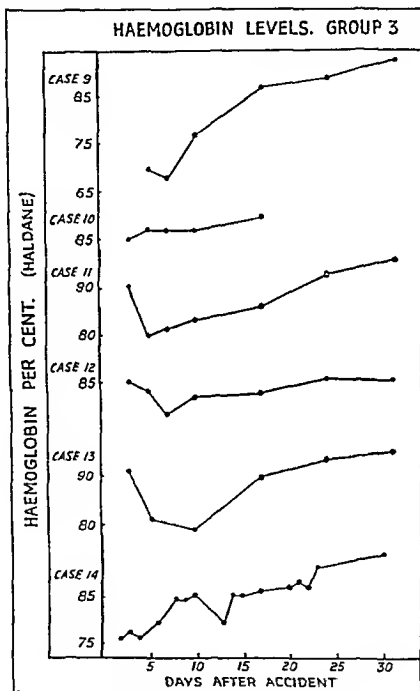
Fig. 5.—Hæmoglobin levels. Group III.

Hæmoglobin and red cells (fig. 5). In four cases the Hb. fell while under observation, reaching the lowest level between the 5th and 10th day after injury and then rising to a figure higher than that seen on first examination. In one patient (case 10) there was a slow rise from the 3rd day after injury, in another a steady rise from the low figure of 76 per cent. on the 2nd day to 94·5 per cent. on the

30th day. The changes in Hb. were roughly parallel to the changes in the total red cell count. No abnormalities were noticed in the appearance of the red cells in stained films.

Mean corpuscular volume. This showed no striking change in any instance while under observation, except in case 14, when on the 4th day a mean corpuscular volume of 99 c.μ was noted, a figure which is above normal limits. It was, however, a single observation and undue importance should not be attached to it.

Reticulocytes. In five cases where the reticulocyte count was not done daily there was a rise in the reticulocytes during the first seven
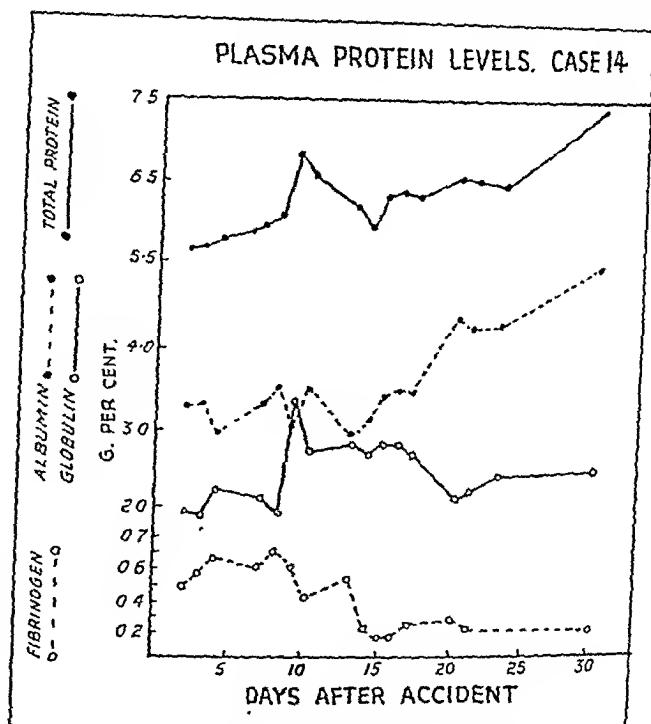
FIG. 6.—Plasma-protein levels. Case 14.

days of observation, followed by a fall to under 0·5 per cent. The maximum observed was 2·1 per cent. In patient 14, on whom counts were done almost daily, the curve is somewhat more irregular but of the same type, *i.e.* a low figure during the first few days of injury, rising and then falling to a figure under 0·5 per cent.

White cells. No constant or striking change in the total white-cell count was found. In 4 cases fluctuations were within normal limits. In two a leucocytosis affecting the polymorphs more particularly was observed on one occasion.

Serum bilirubin. This was followed in 5 patients (cases 9-13). No figure higher than 0·98 mg. per cent. was seen. In all cases the maximum figure was found not more than 6 days after injury, the curve then falling to a figure under 0·4 mg. per cent.

Urobilin. Unfortunately urobilin was only looked for regularly in the urine of case 14. It was present in traces on the 5th day after injury and in considerable amounts for the following 5 days.

Serum protein. Total and differential estimations of plasma protein were made in case 14 (fig. 6). Total proteins only were estimated on cases 9–13 (fig. 7). Two of the latter showed a fall followed by a rise. In two there was a considerable rise from a low level while under observation, and in the fifth there was no significant change from an initial level of 6·0 mg. In case 14, the initial figure was 5·12 g. on
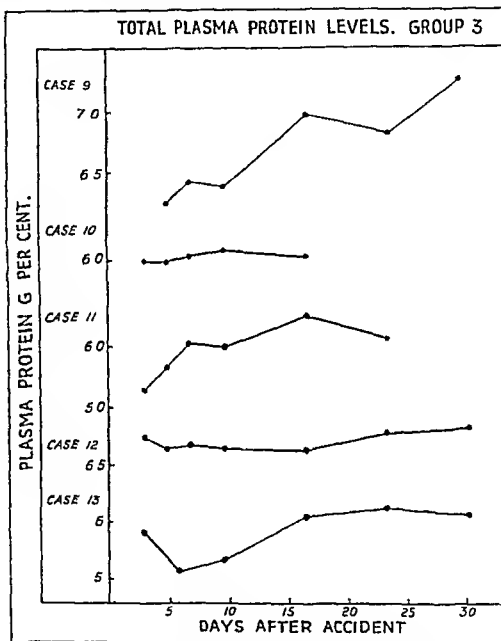
Fig. 7.—Total plasma-protein levels. Group III.

the 2nd day, rising on the 9th day to 6·85 g. The differential estimation showed this rise to be due largely to a rise in globulin and fibrinogen, the albumin at this point being low. This rise was followed by a fall in globulin and fibrinogen and a slow rise in albumin, which however did not compensate for the fall in the other two, so that the total protein fell again until the 14th day, when there was a more steady rise in albumin.

DISCUSSION

Analysis of the observations made on 14 cases of injury show that certain common features were present, though the type of injury was very variable. There was a fall in Hb. during the first week followed by a rise, or a rise from an initial low level. Changes in Hb. were accompanied by parallel changes in the total red-cell count. In only three instances was there a significant increase in red-cell size during the time of the anæmia. No gross abnormality was noticed in the appearance of the red cells in stained films. The reticulocyte count was low immediately after injury, but then rose to a figure within or just above the upper limits of normal, immediately preceding the rise in Hb., and followed by a return to a steady low normal figure. Serum bilirubin showed no rise above normal but higher figures were found in the first few days after injury than at a later date. Urobilin was present in the urine in several cases. Total serum protein either fell immediately after injury, rising again slowly after a few days, or else showed a steady rise from an initial low level. It is, however, probable that the estimation gives a fallacious picture of protein metabolism, as in the three instances in which differential proteins were estimated there was a considerable fall in albumin associated with a rise in globulin and fibrinogen, the rise in the two latter being sufficient in some instances to mask the fall in albumin.

The raised sedimentation rate found was presumably dependant upon the raised level of plasma fibrinogen. The changes noted in the total and differential white-cell counts were in no way striking. No example of leucopenia was encountered. This is known to occur in some instances after burns (Brown, 1944; F. H. L. Taylor, 1945 (personal communication); Vaughan, 1945 (unpublished observation)), even when no sulphonamides are given.

The development of anæmia following injury other than burns has been discussed repeatedly by those handling both battle and air-raid casualties, though it has received little comment in the literature (Freebody, 1943-44). It has, however, been recognised as a serious complication of burns (Cope and Rhinelander, 1943; Vaughan, 1943; Brown, 1944) and has recently been shown to occur after operative interference which is not preceded by injury (Seaman and Ponder, 1943).

Brown (1944) has suggested that the anæmia following burns may be of three types : (i) a phase of temporary anæmia which is maximal about the 5th-7th day and which disappears by the 10th-14th day ; (ii) a phase of rapidly developing and moderately severe anæmia, less readily visible and reaching a maximum in 10-14 days ; (iii) a phase of chronic anæmia, the duration of which corresponds roughly with that of febrile period.

There is no very clear reason for distinguishing between the first two phases. Both types of anæmia are clearly similar to that described

in the present communication and found by Seaman and Ponder after operation. No example of the more prolonged and severe type of anæmia is included in the present series and it is not, therefore, further discussed.

The cause of this early anæmia remains obscure. It has been suggested that it is due (i) to blood loss at the time of injury or operation, (ii) to a hæmolytic process, (iii) to a disturbance of plasma volume following repeated transfusions, (iv) to a disturbance of hæmoglobin metabolism.

The fact that in the majority of cases of both trauma and burns the anæmia is progressive during the first week suggests that blood loss is not alone sufficient to account for it. The anæmia may also occur after simple fracture when, apart from hæmatoma formation, there has been no loss of blood. In the case of the anæmia following operative interference, Seaman and Ponder were able to calculate by direct estimation of the blood loss in swabs and dressings that this was in fact insufficient to account for the fall in Hb.

There is definite evidence (Shen and Ham, 1943) that a hæmolytic process may result from severe burns, since hæmoglobinæmia, hæmoglobinuria and changes in cell shape and fragility may all occur, probably as a result of the actual changes produced in the red cells at the time of burning, which renders them more easily destroyed, rather than to a more prolonged hæmolytic process (Shen and Ham, 1943 ; Brown, 1944). Following trauma the red cells are not subjected to any such insult ; conditions are therefore different. The present observations do not suggest that a hæmolytic process is present. No changes in cell shape were apparent in stained films, and while measurements of red-cell fragility were few, those that were made showed normal fragility. The rise in serum bilirubin and the presence of urobilin in the urine cannot be interpreted as evidence of hæmolysis in the absence of other measurements of pigment excretion and may equally well be indicative of disturbances of hæmoglobin metabolism (Vaughan and Saifi, 1939). Observations on complete pigment excretion can alone determine if a hæmolytic factor is present. The evidence at present available suggests that it is not. It has been suggested that the anæmia may result from disturbances in blood volume, especially in patients receiving massive transfusions of serum or plasma. Since, in the present series, a fall in Hb. occurred in patients receiving no transfusions, such mechanically produced dilution can at most be merely a contributory cause in some cases and not the fundamental ætiological factor. That physiological dilution as suggested by Brown may occur cannot be ruled out, however, without complete blood volume observations made in parallel with the other estimations described here. Simple bed rest in healthy young men unassociated with trauma, however, causes a reduction in blood volume rather than an increase (Keys, 1945), and such dilution seems unlikely. If an increased plasma volume was

general a constant fall in total protein would be expected to accompany the anæmia. Such a fall does not necessarily occur, though there may be remarkable changes in the albumin-globulin ratio.

By a process of exclusion, therefore, it appears that the progressive anæmia in the first ten days following trauma is probably due to a disturbance of hæmopoiesis, as suggested by Seaman and Ponder to explain the anæmia following operative interference. The mechanism of this disturbance is not known. It is true that many of the patients were receiving sulphonamide drugs and that fever was also present. It might be claimed therefore that the disturbance was dependent upon either the drugs or the sepsis (Vaughan and Saifi, 1939; Saifi and Vaughan, 1944), both of which will produce anæmia, probably as a result of interference with the synthesis of hæmoglobin. Again, studies of pigment metabolism are required to throw light upon this hypothesis, but it should be remembered that neither factor was invariably present.

It is tempting to link the changes in the Hb. level with the changes in plasma protein that occur after trauma, since the synthesis of Hb. is closely related to that of plasma protein (Hahn and Whipple, 1939).

Following injury, burns or operative interference there is a reversal of the albumin-globulin ratio and a rise in fibrinogen (Chanutin *et al.*, 1938; Taylor *et al.*, 1943; Elman, 1944; Croft and Peters, 1945). In some cases the fall in albumin is masked by the rise in the two other fractions, so that the total serum protein is unchanged; in others there is also a fall in total plasma protein.

The disturbance in plasma protein level has been shown by other workers to be associated with considerable losses of nitrogen in the urine and a rise in blood urea, leaving the patient or experimental animal in negative nitrogen balance (Cuthbertson, 1944). Croft and Peters have suggested that the increased metabolism of tissue proteins thus indicated is due to the need of the body for one or two particular amino acids; this results in the raiding of the tissue and labile body proteins and the elimination of the unwanted amino acids. The authors in fact have been able to show that in burnt rats methionine exerts a most effective action in relieving this nitrogen loss. It remains to be shown that methionine exerts the same action in other forms of trauma. The need of the body for methionine is possibly associated with its function of methylation (Croft and Peters). Numerous experiments have shown that methionine does exert some protective action on the liver (Messinger and Hawkins, 1940; Miller and Whipple, 1942; Daft *et al.*, 1942; Himsworth and Glynn, 1939-42; Goodell *et al.*, 1944), and the evidence available suggests that both burns and trauma are associated with a disturbance of liver function. There is a fall in serum proteins with a reversal of the albumin-globulin ratio (Higgins *et al.*, 1944), a raised serum bilirubin and excretion of urobilin in the urine (Vaughan and Saifi).

It is possible that as the body stores are raided for methionine they may also be raided for another amino acid—lysine—which is essential for red-cell and hæmoglobin formation (Harris *et al.*, 1943). The increased demand for lysine may be dependent upon the deficient liver function resulting in failure of Hb. synthesis, the demand for methionine being possibly dependent upon altered liver function. The importance of the liver in hæmopoiesis is well recognised though still imperfectly understood. Thus both anæmia and hypoprotein-æmia may be dependent upon disordered liver function.

The fall in serum protein in burns can, to some extent, be checked by massive protein feeding (Taylor *et al.*; Croft and Peters) though the extent to which this may occur has been questioned in the case of fractures (Cuthbertson, 1936). Little is known of its effect upon the anæmia. Case 14 in the present series is of interest in this connection. He was a young man who received multiple injuries as a result of enemy action, among them a perforating wound of the stomach. When seen on the second day he had a low Hb., presumably due to blood loss at the time of the incident, which then, unlike that of other patients, rose steadily. The characteristic changes in plasma proteins were however present. Unlike the other patients, he received from the outset a reasonable protein intake of 56-86 g. daily owing to the initiative of a ward sister. It is suggested that this patient, owing to his protein intake, was able to synthesise Hb., but not enough was given to maintain the serum-albumin.

Further detailed studies, including complete pigment metabolism experiments and experimental feeding with different amino acids are needed in all types of trauma if the mechanism of the many disturbances of metabolism are to be understood. From a practical point of view it would appear essential to ensure a high protein intake for all cases of trauma. Diet in the surgical wards should be regarded as of equal importance in treatment as aseptic technique.

CONCLUSIONS

1. Observations on the blood picture, serum bilirubin, plasma proteins and urinary urobilin have been made on 14 patients following injury.

2. The majority of patients, irrespective of the character of their injury, showed during the first week a fall in hæmoglobin and red cells and either a fall in plasma proteins or an initial low level. When the differential proteins were estimated, albumin was seen to fall but this was accompanied by a rise in globulin and fibrinogen. A rise in hæmoglobin was preceded by a rise in reticulocytes and was associated with a rise in red cells and plasma protein. Though within normal limits, the serum bilirubin was higher during the first few days of observation than subsequently. The excretion of urobilinogen in the urine was noticeable in several cases.

3. It is suggested that the anæmia occurring in the first ten days after injury is not due to blood loss or hæmolysis but to a disturbance of hæmoglobin synthesis dependent upon disordered liver function.

4. The importance of high protein intake in all surgical cases is stressed.

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THE CHANGES IN THE KIDNEYS IN CARBON TETRACHLORIDE POISONING, AND THEIR RESEMBLANCE TO THOSE IN THE "CRUSH SYNDROME"

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(PLATES CXLIX AND CL)

PROFOUND and sometimes fatal involvement of the kidneys in carbon tetrachloride poisoning has been recognised for some time (Dudley, 1935 *a* and *b*), though emphasis has more often been laid on the damage sustained by the liver. The three cases here described were all in young men, serving in the Navy, who became affected after dry-cleaning their clothes in a confined space with Pyrene obtained from fire-extinguishers. Case 1 has already been reported elsewhere (Forbes, 1944) but is included here since no opportunity was then afforded for revision and extension of the original routine report submitted on the specimens sent for histological examination.

The close resemblance observed between the renal changes in this condition and in the "crush syndrome" forms the principal reason for this communication, and a theory accounting for certain features of the histological picture will be presented.

Case 1

Clinical. A French petty-officer, aged 35 years. Apart from his reputation as a heavy drinker nothing was known of his previous history. He was one of a group who took to cleaning their clothes, in a cabin about 25 ft. by 10 ft., about once a week with Pyrene until, some three months later, three of them were taken ill, a few days after their last exposure, with headache, general malaise, backache and vomiting. In the present case the man complained of extreme anorexia, thirst and attacks of double vision which began ten days before admission to hospital. Six days before death he began having epileptiform convulsions and became comatose.

On examination, there was no jaundice and the liver was not palpable. Blood pressure was 160/90 mm. Hg., lumbar puncture yielded normal cerebro-spinal fluid, and blood urea was 143 mg. per 100 ml., rising later to 365 mg. There was heavy albuminuria, with abundant leucocytes and red cells but no casts in the deposit. With the administration of sedatives and intravenous glucoso-saline he became partly conscious, but there was incontinence of urine

and fæces. He developed signs of heart-failure, with gallop-rhythm and pulmonary œdema, and died sixteen days after the onset of the illness and six days after admission to hospital.

Necropsy (Surg.-Commander T. W. Froggatt, R.N.). Intense œdema and congestion of lungs. Dilatation of right side of heart, contraction of left. Cloudy swelling of myocardium. Pus in crypts of fibrosed tonsils. Enlargement of glands of neck. A little yellowish fluid in peritoneal cavity. Nutmeg type of congestion of liver. Kidneys enlarged (left 9·5 oz., right 8·5 oz.) and swollen, with smooth subcapsular surfaces and pale cortex; deep congestion of medulla. Albuminous urine (8 oz.) in bladder. Congestion of meninges but no further abnormality in brain.

Microscopical· examination

Small pieces of kidney and liver and a cervical gland were received in formalin. After paraffin embedding, sections were stained with hæmatoxylin and eosin, iron hæmatoxylin and van Gieson, and Weigert's elastic stain. In addition sections of the kidney were stained with Azan and by the benzidine method for red cells. Frozen sections from the kidney and liver were stained with Sudan III.

Kidney. The glomerular tufts are full but show *no increase of* endothelial cells. The red-cell content of the capillaries is uneven, many of the vessels being empty. The capsular spaces contain considerable quantities of eosinophil flocculent debris. In association with this there is severe albuminous degeneration and desquamation of the capsular epithelium, many areas being denuded. The efferent passage into the first convoluted tubule is often dilated and the epithelium here is swollen and frequently pouts into the adjacent part of the capsular space. Apart from a few fibrosed ischæmic glomeruli the glomerular appearances are otherwise normal.

Throughout the tubules the lumina are somewhat dilated. Severe albuminous and dropsical degeneration affects the epithelium of the first convoluted tubules, but there is no hyaline-droplet degeneration nor necrosis. The lumina contain eosinophil flocculent material similar to that in the glomerular capsules. Many groups of second convoluted tubules and ascending loops of Henle are lined with a greatly flattened epithelium in which the nuclei are unequally spaced and in which considerable lengths are altogether devoid of nuclei. Pyknosis and karyorrhexis are often present. Such tubules frequently contain casts, either of brilliantly eosinophil, fuchsinophil particulate material suggestive of fragmented red corpuscles, or of finely granular less eosinophil material with a faint orange tinge. Mixed with these granular casts, or present in other tubules, are pale eosinophil hyaline casts which stain blue with Azan. Descending towards the medulla, all types of cast occupy the collecting tubules and are more numerous than in the cortex. In the lower medulla almost every tubule contains

PLATE CXLIX

KIDNEYS IN CARBON TETRACHLORIDE POISONING

Fig 1—Case 1   Medullary pyramid showing engorgement of vasa recta and cast in
collecting tubule   H and E   ×230.
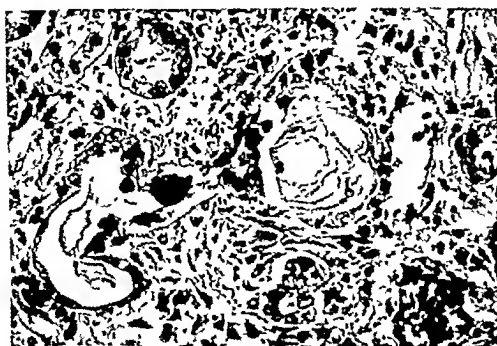
Fig 2—Case 1   Tubulo venous communication in intermediate zone   Tubule on
left, vein on right   H and E   ×310

KIDNEYS IN CARBON TETRACHLORIDE POISONING

Fig 3—Case 1 Tubulo venous communication the site being occupied by a mass of thrombus H and E ×220
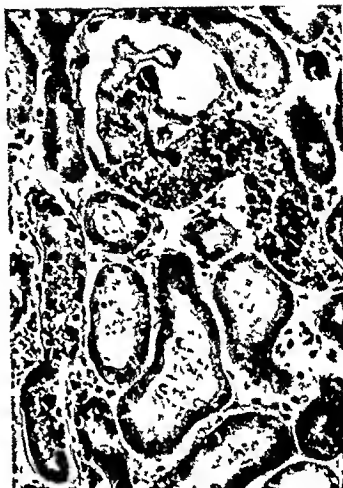
Fig 4—Case 3 Convoluted tubules in lower cortex with particulate matter in two H and E ×230

Fig 5—Case 3 Tubulo venous communication in intermediate zone The tubule is represented by a V shaped clump of epithelial cells at right border of vein H and E ×230

a cast which, in hæmatoxylin and eosin preparations, is pinkish orange and sometimes incorporates brightly eosinophil granules.

There is patchy engorgement of the capillaries in the cortex but great and uniform engorgement of the vasa recta (fig. 1). Some large veins adjacent to the arcuate arteries and their branches in the intermediate zone are dilated, and many contain portions of mural thrombus. Such areas of thrombosis are often closely contiguous to an adjacent renal tubule, apparently in the ascending loop of Henle, and in several such sites a communication can be demonstrated between the two lumina (figs. 2 and 3). The epithelium of the tubule then shows a disorderly arrangement, with occasional heaping up of the cells upon one another and evidence of proliferation in the form of karyokinesis. Groups of epithelial cells are often seen in the mass of thrombus coating the wall of the adjacent vein. Occasionally fresh red corpuscles are present in tubules near the site of such communications. In benzidine preparations these cells give the same grade of reaction as do the intravascular red cells, but the brightly eosinophil particulate matter in the renal tubules elsewhere gives a fainter reaction.

There is a patchy formation of young granulation tissue in the intermediate zone in relation to these tubulo-venous communications. In the medulla there is often focal infiltration of the tissues around the engorged vasa recta with small lymphocytes, plasma cells and cells indistinguishable from hæmocytoblasts. Occasionally there are small recent hæmorrhages in these areas. The blood within the adjacent vasa recta includes a considerable number of immature white cells, including hæmocytoblasts. Elsewhere there is slight hypertrophy of the intima of the arteries, but no definite medial hypertrophy. In the frozen sections there is no sudanophil fat in the kidney.

Liver. A small area in the centre of each lobule shows severe degeneration and necrosis, with very little associated fatty change. There are also cells containing pigment which gives a positive reaction for iron. In these areas the sinusoids are dilated, but this seems to be secondary to the necrosis of liver cells, because it is not found in the intermediate zones of the lobules. In the centres of the lobules and in the portal systems there is slight infiltration with lymphocytes and larger mononuclear cells. The liver parenchyma elsewhere appears normal.

Cervical lymph gland. There is conspicuous sinus catarrh.

Case 2

Clinical. A lieutenant R.C.N.R., aged 34 years, was admitted to hospital four days after he had cleaned clothes with Pyrene in an enclosed compartment on board ship. He complained of vomiting and of pain in the back and abdomen. On examination the urine contained albumin, with debris, leucocytes and a few red cells in the deposit. No red cells were found four days after admission but

albuminuria was persistent. Seven days after admission the blood urea was 266 mg. per 100 c.c. Blood count :—red corpuscles 4,880,000, Hb. 102 per cent., white cells 13,200 (neutrophil polymorphonuclears 70, lymphocytes 18, mono nuclears 8, eosinophil polymorphonuclears 3 and basophils 1 per cent.). He died, apparently in uræmia, thirteen days after exposure.

Necropsy (Surg.-Commander T. W. Froggatt, R.N.). Heart distended with fluid blood. Myocardium pale and flabby. Almost complete obliteration of pleural sacs by fibrous adhesions; a little free fluid in remainder of cavities. Intense œdema and congestion of lungs. Small amount of clear fluid in peritoneal cavity. Liver (4 lb. 10 oz.) slightly swollen, with mottled cut surface due to congestion of centres of lobules. Spleen (10½ oz.) congested, with hyperplasia of pulp. Kidneys (right 8½ oz., left 8 oz.) swollen and friable, with very pale cortex. Congestion of pancreas, stomach and small intestine.

Microscopic examination

Pieces of kidney, liver, pancreas, suprarenal, spleen and lung were received in formalin. The same technique was used as in case 1.

Kidney. The appearances are closely similar to those described in case 1 except in a few particulars. The lumen of one tubule in the cortex, probably second convoluted, is filled with neutrophil leucocytes. In other convoluted tubules there are occasional spheroids of crystalline appearance, with radial striation, that are either colourless or faintly hæmatoxyphil. Apart from the abundant casts, of a similar character to those in case 1, there are collections of hæmatoxyphil amorphous material which are sometimes invested by a layer of flattened epithelial cells. The abundant granular casts in the collecting tubules in the medulla are lightly eosinophil or more often faint pinkish brown. There are no demonstrable thrombi in the veins nor can tubulo-venous communications be clearly seen, but changes are present in the interstitial tissue of a similar character to those present about communications in case 1. In addition there is sparse focal infiltration of the cortex with small lymphocytes and occasional neutrophil leucocytes. A similar infiltration, including eosinophil leucocytes, is present about the large veins of the intermediate zone.

Liver. The appearances are similar to those in case 1. Early stages of regeneration are present in the liver parenchyma about the central areas of necrosis in the lobules. Large droplets of sudanophil material are present in occasional cells in the less affected parts of the parenchyma.

Spleen. There is great engorgement of the pulp. A moderate number of myelocytes and hæmocytoblasts are diffusely distributed in the strands and sinusoids. *Lung.* There is diffuse engorgement and slight anthracosis. *Suprarenal and pancreas* normal.

Case 3

Clinical. A supply assistant, R.N., aged 26 years, was admitted to hospital with general malaise, backache and vomiting of three days' duration, coming on 4 days after he had cleaned his clothes with Pyrene. He had passed very little urine during the three days before admission and it had been found to be loaded with albumin.

On examination, he was pale and rather puffy under the eyes. Subconjunctival hæmorrhages present in both eyes. Slight pitting œdema about the ankles. Blood pressure 154/86 mm. Hg. Apart from tenderness over the kidneys the abdomen was normal. Blood urea 170 mg. rising to 347 mg. per 100 ml. two days before death. The urine yielded a dense cloud of albumin; numerous red corpuscles and leucocytes in deposit. Hijmans van den Bergh reaction negative; icterus index 4.

Five days after admission he developed pulmonary œdema. The output of urine fell to 1·5 oz. on the day before death and he died, after increasing difficulty in respiration and the expectoration of blood-stained frothy sputum, on the eleventh day of illness and fifteen days after exposure to carbon tetrachloride.

Necropsy (Surg.-Commander W. P. E. McIntyre, R.N.). Dilatation of left auricle. Blood-stained frothy fluid in trachea and bronchi. A few fibrous pleural adhesions over posterior surface of left lung. Intense œdema and congestion of lungs. A small amount of clear fluid in peritoneal cavity. Congestion of centres of lobules of liver (58 oz.). Spleen normal. Kidneys (right 8 oz., left 8½ oz.) swollen, with pale cortex. Congestion of small intestine.

Microscopic examination

Three paraffin blocks of portions of kidney, liver and lung respectively were received. . The stains used were the same as in case 1.

Kidney. The appearances are identical with those in case 2 except in the following details. The amount of albuminous debris in the glomerular capsules is rather less in the present case. The predominance of brightly eosinophil particulate casts is more accentuated, both in cortex and medulla (fig. 4); they are intimately mixed up, in places, with desquamated epithelial cells and albuminous eosinophil material and many of the casts are coated with epithelium. One tubulo-venous anastomosis was identified (fig. 5) without thrombosis in the affected vein, but in the photograph the details of the tubular element of the lesion are obscure.

Liver and lung. The appearances are similar to those in case 2.

DISCUSSION

The clinical and pathological picture in these three cases is remarkably uniform. Vomiting, malaise and pain in the abdomen and back followed exposure to Pyrene. There was no record of jaundice, and hæmorrhages were noted only in case 3, where the conjunctivæ were affected. On the other hand renal function was obviously dis-

turbed. The output of urine was greatly diminished (case 3), though not suppressed to the point of anuria; in cases 1 and 2 the output was not measured, but in case 1 there was coma and incontinence of urine and fæces. The blood urea was greatly raised in all cases, but it is of interest that the still higher level of 746 mg. was noted by Forbes in his case 2 and was compatible with recovery. The urine in these cases contained large amounts of albumin; red corpuscles and leucocytes were abundant in the deposit in cases 1 and 3 and less numerous in case 2. In cases 1 and 3 heart failure with pulmonary œdema appears to have been an outstanding terminal development.

Pathologically it seems clear that the renal and not the liver damage was responsible for death. In the liver, necrosis is confined to the centres of the lobules; the remainder of the parenchyma appears normal and there is evidence of early regeneration about the central foci in cases 2 and 3. The liver therefore appears to have entered a stage of repair.

In the kidneys, on the other hand, there is microscopical evidence of severe disturbance. The histological picture is indistinguishable from that found in the crush syndrome: the severe degeneration, with loss of cells and evidence of early regeneration in the second convoluted tubules and ascending limbs of Henle, the abundant casts seemingly derived from fragmented red corpuscles, together with the characteristic orange-brown or café-au-lait pigmented casts, are features common to the two conditions. In particular, attention is drawn to the tubulo-venous communications observed in cases 1 and 3. These were first described by Shaw Dunn, Gillespie and Niven (1941) in cases of crush syndrome and have been confirmed by subsequent workers. Prolonged search failed to reveal such lesions in case 2 and one only was identified in case 3, possibly because no venous thrombi were present to act as pointers to the sites. Other histological features, notably the focal interstitial reaction in the boundary zone between cortex and medulla, suggested that sites of communication were eluding discovery. The fact that in case 1 survival after exposure to " Pyrene " was somewhat longer than in cases 2 and 3 may mean that the stage of development of venous thrombosis had not been attained by the latter cases. In a case of crush syndrome which came to necropsy in this department (P.M. 62, 1945) thirty-six hours after injury no tubulo-venous communications could be identified. Though Shaw Dunn implied that these communications were formed by a break-through from the tubule into the vein, a view that has been endorsed by Bywaters and Dible (1942), it is here suggested that the pressure may be exerted in the opposite direction. If so, these lesions are to be regarded as of fundamental importance in the development of the renal picture as a whole. It has recently been demonstrated (Barclay *et al.*, 1946-47) that in the crush syndrome there is a shunting of the renal circulation from the cortex to the medulla, apparently from reflex nervous impulses. The blood is thus

diverted into the vasa recta which form the arterio-venous anastomoses. As a result, the cortex suffers anoxia and the medullary vessels are greatly engorged. It may follow that the sudden altering of the relative pressures in the veins and tubules in the medulla leads to rupture where the walls of these structures are in close apposition, with the entry of blood into the lumina of the tubules. Such a hypothesis would account well both for the presence of red cells in the urine and for the distribution of the casts which appear to be derived from red cells in the loops of Henle, second convoluted and collecting tubules, and the immunity of the first convoluted tubules. For there seems little ground for the suggestion of Bywaters and Dible that these peculiar casts are formed by the condensation, owing to loss of water, of the flocculent debris higher up in the nephron. Were this so it might be expected that such a transition would frequently be seen in examples of severe parenchymatous degeneration of the kidney in general. This is not our experience. Unless blood escapes into the tubules in the manner here suggested, histological examination of the kidneys points to no obvious alternative source. Further support for this theory is found in the fact that fresh red corpuscles are identifiable within the tubules only at points near the sites of these tubulo-venous communications. If the theory is correct, then the stage represented in case 1, in which mural venous thrombi are present at the sites of communication, should be regarded as a stage of healing in which the breach in the wall is being sealed by thrombus on the one side and by epithelial proliferation on the other.

SUMMARY

Three cases of carbon tetrachloride poisoning are described in adult men in whom death was attributable to severe changes in the kidneys. These changes resemble those characteristic of the " crush syndrome ". It is suggested that the tubulo-venous communications present may be largely responsible for the clinical and histological features of this type of renal disease.

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